

**HUMAN INFORMATION PROCESSING DURING ACUTE
INSULIN-INDUCED HYPOGLYCAEMIA**

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To Dorothy for being there.

DECLARATION

- a) This thesis was composed by Rory J. McCrimmon.
- b) The studies presented were all performed, analysed and written by myself with the exception of *Study 5*; 3 of the 6 studies from which hypoglycaemia symptom data had been collated for further analysis in this study were conducted by other investigators (referenced in text). I performed the statistical analysis of the collated data.
- c) I hold the degree of M.B. Ch.B. (Edinburgh).
- d) This thesis has not been submitted for any other degree, diploma, or professional qualification.

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ABSTRACT OF THESIS

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Acute insulin-induced hypoglycaemia in humans is known to interfere with cognitive function through the effect of neuroglycopenia on the brain. However, the effect of acute hypoglycaemia on specific brain functions remains largely unexplored. The literature relevant to the effects of acute and recurrent hypoglycaemia on general cognitive processes is reviewed. The research described in this thesis includes studies involving experimentally induced hypoglycaemia, and a clinical study of children with type 1 diabetes.

The effect of acute insulin-induced hypoglycaemia on basic sensory information processing was examined. The principal studies performed were: *Study 1* - an examination of the effect of acute hypoglycaemia on visual information processing, and; *Study 2* - an examination of the effect of acute hypoglycaemia on auditory information processing. During 60 minutes of exposure to moderate hypoglycaemia significant impairments of both visual and auditory information processing were documented. It was also noted that hypoglycaemia did not significantly impair visual acuity for high contrast visual images but did disrupt ability to distinguish between low contrast visual images.

Acute hypoglycaemia induces, in general, a negative mood state. The effect of hypoglycaemia on mood and emotion in human subjects was further explored. Hypoglycaemia was found to induce a small, but significant increase in anger-state that could not be predicted from measures of anger-trait or general anger-expression (*Study 3*). Hypoglycaemia also induced a negative mood-state characterised by increased tension and decreased happiness, lead to more negative appraisals of a life event, but did not effect a change in personality traits (*Study 4*).

In addition, studies of were performed on different aspects of the symptomatic response to hypoglycaemia. These included: the symptoms of hypoglycaemia induced by different experimental models of hypoglycaemia (*Study 5*); and the symptoms of hypoglycaemia reported by children with type 1 diabetes (*Study 6*). The symptoms generated during hypoglycaemia induced by either the one-step hyperinsulinaemic glucose clamp or a controlled insulin-infusion do not differ substantially in their frequency, intensity or latent structure. Children with type 1 diabetes differ from adults in their reporting of the symptoms of hypoglycaemia by describing more symptoms indicative of behavioural change.

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LIST OF ABBREVIATIONS

ALE – Appraisal of Life Events Scale
AVLT – Auditory Verbal Learning Test
AX – Anger Expression
AH4 – Alice Heim 4 Test
BAEP – Brainstem Auditory Evoked Potential
BVRT – Benton Visual Retention Test
CFFT – Critical Flicker Fusion Threshold
CRT – Choice Reaction Time
CS – Contrast Sensitivity
DSS – Digit Symbol Substitution Test
EHS – Edinburgh Hypoglycaemia Scale
ERP – Event Related Potential
EEG - Electroencephalogram
EPQ-R - Eysenck Personality Questionnaire - Revised
IT – Inspection Time
LED – Light Emitting Diodes
MFFT – Matching Familiar Figures Test
DRT – Nelson Denny Reading Test
NART – National Adult Reading test
PASAT – Paced Auditory Serial Addition Test
PCA – Principal Components Analysis
RVIP – Rapid Visual Information Processing
SACL – Stress Arousal Check List
SAT – Scholastic Aptitude Test
SRT – Simple Reaction Time
TM-B – Trail Making Test B
TBAC – Test of Basic Auditory Capabilities
VCD – Visual Change Detection
VMD – Visual Movement Detection
VA – Visual Acuity
VEP – Visual Evoked Potential
VRT – Visual Reaction Time
WAIS-R – Wechsler Adult Intelligence Scale-Revised
UMACL – UWIST Mood Adjective Check List

PART I

INTRODUCTION

CHAPTER 1

HYPOGLYCAEMIA

The introduction of insulin in the 1920's to treat type 1 diabetes transformed the management of this disorder. However, as early as 1922, Elliot Joslin observed that insulin is "...a potent preparation, alike for evil and for good", emphasising that it is potentially a dangerous drug. These early observations stimulated researchers to examine in more detail the clinical and physiological aspects of hypoglycaemia. In the following section the literature on the frequency of hypoglycaemia in the insulin-treated population, risk factors for its development, and its morbidity, will be briefly reviewed. I will then review our current understanding of the physiological mechanisms involved in the hypoglycaemic response. Finally, I will review our current knowledge of the effects of both acute and chronic hypoglycaemia on cognitive function. These reviews provide the historical background that lead to the development of this thesis.

Frequency of hypoglycaemia

The true frequency of hypoglycaemia in the diabetic population is difficult to estimate, and is probably much higher than is documented by individual patients. This is related to the frequent occurrence of asymptomatic biochemical hypoglycaemia (only identified by blood glucose testing), the common clinical problem of loss of awareness of hypoglycaemia, and nocturnal hypoglycaemia, which is often undetected, during sleep. Considerable variation in estimated rates of severe hypoglycaemia exists between published studies because of differences in definition, methodology of ascertainment, and heterogeneity of the patient populations. Recent studies have estimated the frequency of *severe* hypoglycaemia

as this is a more consistent, robust, and clinically relevant measure with respect to the morbidity of this common therapeutic side effect. The cardinal factor in the definition of severe hypoglycaemia is the need for external assistance to resuscitate the patient, irrespective of how low the blood glucose concentration falls or the method of resuscitative treatment. This definition has now received wide consensus.

When severe hypoglycaemia is defined as an episode that the patient is unable to self-treat, retrospective studies of the frequency of severe hypoglycaemia have produced consistent estimates of around 1.1 - 1.6 episodes /patient /year (MacLeod et al. 1993; Pramming et al. 1991; Reichard et al. 1991). In a one year prospective study from Edinburgh 31 type 1 diabetic patients with normal symptomatic awareness of hypoglycaemia had a frequency of severe hypoglycaemia of 0.48 episodes /patient /year (Gold et al. 1994b). Interestingly, 60% of the episodes of severe hypoglycaemia in this group occurred between the hours of 2400 and 0800. Similarly, in a 6 month prospective study of an unselected group of type 1 diabetic patients (n=78) the frequency of severe hypoglycaemia was reported to be 1.6 episodes /patient /year (Cox et al. 1994).

Recently some large prospective studies have been reported which have provided important information regarding the frequency of severe hypoglycaemia. In the Stockholm Diabetes Intervention Study (Reichard and Pihl, 1994) 102 type 1 diabetic patients were randomised to receive either intensified therapy (n=48) or conventional therapy (n=54). This study was designed to assess the impact of lowering the prevailing HbA1c concentration on diabetic microangiopathy. Subjects also reported all episodes of severe hypoglycaemia that had occurred in the period of time preceding each assessment (every 4 months). Severe hypoglycaemia occurred with a frequency of 1.1 episodes /patient /year with intensified therapy and 0.4 episodes /patient /year with conventional therapy over the 7.5 years of follow-up (Reichard and Pihl, 1994).

Findings from a much larger trial, the Diabetes Control and Complications Trial (DCCT) (The Diabetes Control and Complications Trial Research Group, 1991; The Diabetes Control and Complications Trial Research Group, 1997), have recently been reported. Again this trial was principally designed to assess the impact of intensive insulin therapy with multiple injection regimens or continuous subcutaneous insulin infusion devices on diabetic microangiopathy. Prompted by the observation of a three-fold increase in the frequency of severe hypoglycaemia with intensified vs. conventional therapy in the feasibility phase of the study (The Diabetes Control and Complications Trial Research Group, 1987), the DCCT investigators conducted a detailed investigation of the factors associated with hypoglycaemia based on 817 type 1 diabetic subjects followed up for an average of 21 months. The definition of severe hypoglycaemia used in the DCCT was " an event with symptoms consistent with hypoglycaemia, in which the patient needed the assistance of another person and which is associated with a blood glucose concentration of < 2.8 mmol/L or prompt recovery after oral carbohydrates, intravenous glucose or glucagon administration" (The Diabetes Control and Complications Trial Research Group, 1991). The DCCT reported a rate of severe hypoglycaemia of 0.61 episodes /patient /year with intensified therapy and 0.19 episodes /patient /year with conventional therapy. The cumulative incidence of those subjects who experienced their first episode of severe hypoglycaemia over the duration of the trial was 73% with intensified therapy and 41% with conventional therapy (The Diabetes Control and Complications Trial Research Group, 1997). Interestingly, the frequency of severe hypoglycaemia after the first year of the study remained relatively constant with an annual incidence of ~25% with intensified therapy despite the subjects becoming more familiar with this form of management. Certain groups seemed particularly prone to hypoglycaemia and it was of note that 82% of the adolescent type 1 diabetic patients had experienced at least one episode of severe hypoglycaemia during the trial period.

Some difficulties in estimating the frequency of severe hypoglycaemia are highlighted by appraisal of the results of the DCCT (The Diabetes Control and Complications Trial Research Group, 1997). The patients who were recruited for the DCCT were young (age <40 years), had diabetes of relatively short duration (<15 years), were highly motivated and were supervised intensively by a dedicated specialist team. Patients who had significant microvascular disease were excluded, as were those who had experienced more than two episodes of hypoglycaemic coma without warning symptoms in the two years preceding enrolment. The application of these exclusion factors will have removed many patients who would be most at risk of severe hypoglycaemia. It is likely, therefore, that by studying this highly selected group of patients the results of the DCCT are not typical of the frequency of severe hypoglycaemia observed in an unselected diabetic population. This premise is supported by the much higher estimates reported both in retrospective and prospective studies in Northern Europe.

In 1997 the results of a multi-centre uncontrolled prospective German study on the frequency of severe hypoglycaemia with intensive therapy was reported (Bott et al. 1997). A group of 636 type 1 diabetic patients were followed up for a total of 6 years. Unlike the DCCT, subjects were not excluded if they had experienced severe hypoglycaemia prior to study entry, and were only excluded if they were aged over 40 years and had advanced diabetic complications. This group reported a frequency of severe hypoglycaemia which decreased from 0.28 episodes /patient / year prior to entry into the trial to 0.17 episodes /patient /year in the follow-up period (Bott et al. 1997). This is a figure that is considerably lower than that reported by the DCCT, but the definition of severe hypoglycaemia was different. The German group only recorded events in which glucagon or intravenous glucose was given. In the DCCT the rate of coma /seizure reported with intensified therapy was 0.16 episodes /patient /year, which is comparable. In the German study the incidence of severe

hypoglycaemia decreased with time whereas in the DCCT it remained constant, despite additional training designed to avoid hypoglycaemia. This discrepancy may have arisen because overall HbA1c actually rose during the duration of the trial (Bott et al. 1997), and because subjects were seen much less frequently (at 1,2,3 and 6 years of follow-up) and therefore the reliability of reporting may not have been as good. Comparison of retrospective and prospective estimates of severe hypoglycaemia has demonstrated the validity of retrospective assessment over 12 months only (Pramming et al. 1991).

It is very difficult to estimate with accuracy the frequency of episodes of mild hypoglycaemia or the frequency of asymptomatic hypoglycaemia unless they are estimated prospectively (Pramming et al. 1991). The relationship between actual blood glucose concentrations and subjective symptoms of hypoglycaemia is tenuous (Pramming et al. 1990). Nocturnal hypoglycaemia is also very common, although often unrecognised (Lerman and Wolsdorf, 1988). Using mathematical modelling, Thorsteinsson et al. (1986) has shown that a diabetic patient with good glycaemic control might be expected to have blood glucose <3.0 mmol/L every second day.

Risk factors for hypoglycaemia

The causes of hypoglycaemia are protean (e.g. changes in insulin sensitivity and bioavailability, change in insulin pharmacokinetics, inadequate carbohydrate exchange, co-existing endocrine disease). Most episodes can probably be attributed to errors in the management of diabetes which is not surprising given the difficulties of the daily balancing act undertaken by the diabetic patient treated with insulin. Sleep is also a potential risk factor as symptomatic awareness is obtunded during sleep. Pregnancy, with its attendant push to normalise blood glucose levels, carries a higher risk of severe hypoglycaemia and it has recently been reported that 71% of pregnant type 1 diabetic women will experience severe hypoglycaemia during the

gestational period (Rosenn et al. 1995). In the DCCT, nocturnal hypoglycaemia was responsible for nearly half of all documented episodes and 70% of episodes of severe hypoglycaemia occurred between midnight and 0800 a.m. (The Diabetes Control and Complications Trial Research Group, 1997). Hypoglycaemia unawareness is a particularly important risk factor and may underlie many episodes in which no cause is apparent. In a prospective study in our own centre, the frequency of severe hypoglycaemia was found to be six times higher in type 1 diabetic patients who had a history of diminished awareness of hypoglycaemia than in those who had normal awareness (Gold et al. 1994b).

Analysis of the DCCT (The Diabetes Control and Complications Trial Research Group, 1997) has provided further information. Those subjects who experienced severe hypoglycaemia tended to be male, have longer duration of disease, have a lower stimulated C-peptide, a higher daily dose of insulin and a lower HbA1c (The Diabetes Control and Complications Trial Research Group, 1997). Interestingly, a proportional hazards model found that only about 7.4% and 2.4% of the variance in the risk for experiencing a first episode of severe hypoglycaemia with conventional and intensive therapy respectively could be explained when all the factors listed above were included in the model. When a multiplicative intensity model was used to explore the relationship between 'current' HbA1c as a function of time and other factors simultaneously in the risk of severe hypoglycaemia, then 12% and 6% of the risk of severe hypoglycaemia with conventional and intensive therapy respectively could be explained. The main predictive variables were prior number of episodes of severe hypoglycaemia and current HbA1c. Similar findings were reported by Bott et al. (1997) in their multicentre study of type 1 diabetic patients. Patients who experienced severe hypoglycaemia during the period of the study had on average longer disease duration, a lower C-peptide, a lower HbA1c and a higher total daily insulin dose (Bott et al. 1997). It was also suggested in this trial that those subjects who experienced severe hypoglycaemia had lower coping abilities than those who

did not, although the absolute values reported (15.3 ± 3.9 for those who had experienced severe hypoglycaemia versus 14.6 ± 3.6 for those who had not; with a high score indicating low coping abilities) suggest that this would explain a tiny proportion of the variance in the risk of severe hypoglycaemia.

Recently, Cox et al. (1994) have suggested that the variability in daily blood glucose monitoring was more predictive of severe hypoglycaemia than HbA1c. Seventy-eight type 1 diabetic subjects were enrolled in a prospective trial to determine risk factors for severe hypoglycaemia. Subjects were asked to record 50 finger-prick blood glucose recordings over a 2-3 week period and record these on a hand held computer. Episodes of severe hypoglycaemia over the next 6 months were then recorded. Those subjects who recorded both more variable, and lower, blood glucose recordings suffered more frequent episodes of hypoglycaemia. The authors also produced a measure called the blood glucose index (BG index). A detailed explanation of the BG index can be found in the paper, but essentially it reflects the relative number and extent of low blood glucose readings. The authors found that by including the BG index and the standard deviation of blood glucose readings for the subjects in their cohort in a regression model then 43.5% of the variance in severe hypoglycaemia episodes could be accounted for (Cox et al. 1994).

Table.1.1.1. Risk factors for the development of severe hypoglycaemia

FACTOR	STUDY
Male	DCCT 1997
Type 1 diabetes of long duration	DCCT 1997; Bott et al. 1997
Lower glycated haemoglobin	DCCT 1997; Bott et al. 1997; Pramming et al. 1990
Lower stimulated C-peptide	DCCT 1997; Bott et al. 1997
Higher insulin dose	DCCT 1997; Bott et al. 1997; p
Previous severe hypoglycaemia	DCCT 1997; Bott et al. 1997
Low blood glucose index	Cox et al. 1994
Greater standard deviation in daily blood glucose readings	Cox et al. 1994
Hypoglycaemia unawareness	Gold et al. 1994; Clarke et al. 1995; MacLeod et al. 1994
Impaired hypoglycaemia hormonal counterregulation	White et al. 1983; Bolli et al. 1984
Sleep	e.g. Lermann et al. 1988; Bendtson et al. 1988 and 1991; Pramming et al. 1990; Gold et al. 1994; DCCT 1997
Pregnancy	Rosenn et al. 1995
Not carrying emergency carbohydrates	Bott et al. 1997

Symptoms of hypoglycaemia

It has been recognised since the introduction of insulin in the 1920s that 'a characteristic train of symptoms' occurs when the blood glucose falls to beneath the normal range. These symptoms were so typical of a low blood glucose that they were termed the 'hypoglycaemic reaction' (Fletcher and Campbell, 1922). It was also recognised that individuals did not always experience the same symptom complex and that some diabetics could progress to severe hypoglycaemia without experiencing any symptoms at all. Hypoglycaemia unawareness was recognised as long ago as 1922 (Joslin et al. 1922). This issue was highlighted with the introduction of human insulin in the 1980s. A group in Switzerland suggested that the symptoms of hypoglycaemia induced by human insulin may be different from those induced by animal insulin (Teuscher and Berger, 1987; Berger and Althaus, 1987; Berger et al. 1989). A recent review of all the studies, clinical and experimental, that went on to formally examine this issue concluded that there was in fact no evidence to support this claim (Nellemann Jorgenson et al. 1994). However, the controversy did highlight the need to measure symptoms with greater accuracy and resulted in a considerable body of research into the aetiology and classification of hypoglycaemia symptoms.

The symptoms of hypoglycaemia can essentially be subdivided into three groups; autonomic, neuroglycopenic and malaise symptoms. Autonomic symptoms result from the activation of the autonomic nervous system producing stimulation of the sympatho-adrenal system (Cannon et al. 1924) via centres in the hypothalamus (Benzo, 1983). Neuroglycopenic symptoms relate to the direct effect of glucose deprivation on the central nervous system, in particular cortical function, and malaise symptoms are those which prove difficult to allocate to either of the other groups. Objective allocation of appropriate symptoms into these subgroups has come from both experimental and prospective clinical studies. Principal components

analysis (a statistical technique whereby a large number of variables can be reduced to a number of groups where variables tend to associate with each other more strongly) of symptoms reported during experimental hypoglycaemia by 55 subjects with and without type 1 diabetes (Hepburn et al. 1991a) allocated with confidence 5 symptoms to an autonomic grouping and 6 symptoms to a neuroglycopenic grouping. A subsequent retrospective study of 295 insulin-treated diabetic subjects suggested that there might be 5 groups of hypoglycaemic symptoms (autonomic, neuroglycopenic, general malaise, motor and sensory dysfunction) (Hepburn et al. 1992). However, the authors of this study felt a three factor solution might be more appropriate and they repeated the analysis of this original cohort prior to performing confirmatory factor analysis in a second cohort of 303 insulin-treated subjects (Deary et al. 1993b). Confirmatory factor analysis showed that the three-factor model was the optimal model for explaining symptom covariance in each group (Deary et al. 1993b). This classification is shown below:

Table. 1.1.2. Edinburgh Hypoglycaemia Scale (Deary et al. 1993b).

AUTONOMIC	NEUROGLYCOPENIC	MALAISE
Sweating	Confusion	Nausea
Palpitation	Drowsiness	Headache
Shaking	Odd behaviour	
Hunger	Speech difficulty	
	Incoordination	

There is considerable agreement between the findings from analysis of symptom reports and from experimental studies. For instance, physiological tremor (shaking) is recognised to increase during hypoglycaemia (Heller et al. 1987) and is similarly increased by an infusion of adrenaline (Fellows et al. 1986). Tremor can be suppressed during hypoglycaemia by adrenergic and parasympathetic blockade

(Towler et al. 1993) indicating a role both for adrenaline released from the adrenal medulla into the circulation, and for noradrenaline released from sympathetic postganglionic nerve terminals in relevant target organs. These findings are consistent with patient self-reports, which clearly identify tremor as an autonomic symptom. Sweating is mediated by cholinergic sympathetic nerve fibres and increases dramatically during experimental hypoglycaemia (Corrall et al. 1983; Hepburn et al. 1991a). The sweating response is absent in tetraplegic (sympathectomized) individuals (Frier et al. 1981) but present in those who have had an adrenalectomy (Altorfer et al. 1981). Sweating is also unaffected by beta blockade (Kerr et al. 1990), or by combined alpha- and beta-blockade (Towler et al. 1993), but can be blocked by atropine (Towler et al. 1993). The hypoglycaemia-induced sweating response is therefore sympathetic cholinergic nerve fibre stimulated and can be allocated to the autonomic symptom subgroup on the basis of clinical and experimental studies. Similarly, panautonomic blockade during experimental hypoglycaemia reduces the intensity of classical autonomic symptoms but has little effect, or even increases awareness, of neuroglycopenic symptoms such as drowsiness and confusion (Towler et al. 1993). This again is consistent with patient self-reports, which identify these symptoms as neuroglycopenic.

However, differences do exist between the symptoms reported during experimentally induced hypoglycaemia and hypoglycaemia experienced in the community. This is, in many ways, not surprising given the nature of hypoglycaemia in the two different environments (one taking place in a controlled laboratory environment, the other in every day life with a multitude of other influences). Probably the most detailed analysis of experimental hypoglycaemia to date is that of Towler et al. (1993) who examined 10 non-diabetic subjects using a one-step hyperinsulinaemic glucose clamp. All subjects underwent 4 study sessions (euglycaemia vs. hypoglycaemia vs. hypoglycaemia plus adrenergic blockade vs. hypoglycaemia plus panautonomic blockade). The authors were able to show a clear

physiological basis for the generation of autonomic symptoms (e.g. tremor principally adrenergic; sweating principally cholinergic) and that neuroglycopenic symptoms were unaffected by panautonomic blockade. The authors found that symptoms of hypoglycaemia-associated malaise (nausea and headache) did not change significantly during hypoglycaemia. The particular symptoms of neuroglycopenia that were found to increase in this study also differed from the Edinburgh studies (Hepburn et al. 1991a; Hepburn et al. 1992; Deary et al. 1993b) in that warmness and weakness were found to be neuroglycopenic symptoms (i.e. not affected by pan-autonomic blockade) rather than either autonomic or non-specific. In fact, in the later analysis by Deary et al. (1993) both warmness and weakness were excluded from the Edinburgh Hypoglycaemia Scale because they showed poor discrimination. The classification proposed by Towler et al. (1993) for experimental insulin-induced hypoglycaemia is shown below.

Table. 1.1.3. Symptoms of acute insulin-induced hypoglycaemia under experimental conditions (Towler et al. 1993).

NEUROGENIC SYMPTOMS	NEUROGLYCOPENIC SYMPTOMS
Sweaty	Warm
Hungry	Tired /drowsy
Shaky/tremulous	Weak
Heart pounding	Difficulty thinking /confused
Tingling	
Nervous /anxious	

Morbidity of hypoglycaemia

Most obviously severe hypoglycaemia, and the reduced conscious level that results from significant neuroglycopenia, is associated with the risk of trauma and injury to an individual or to others as a result of their activities. Fractures of long bones, joint dislocations, soft tissue injuries, head injuries and burns have been described in association with acute hypoglycaemia (Hepburn et al. 1989) and hypoglycaemia while driving can cause road-traffic accidents (Frier, 1992). Various forms of trauma are associated with hypoglycaemia-induced convulsions, which affect about 7% of all insulin-treated patients (MacLeod et al. 1993). However, more widespread effects of hypoglycaemia have also been reported and these will be discussed in the following section.

Cardiovascular system

Acute hypoglycaemia in diabetic patients has been associated with various major vascular events such as stroke and myocardial ischaemia/infarction although much of the evidence is anecdotal. The cardiovascular morbidity of hypoglycaemia may be secondary to profound sympatho-adrenal stimulation or as a consequence of the concurrent haemodynamic and rheological changes.

Anecdotal reports have been described of hypoglycaemia-induced myocardial ischaemia or infarction (Fish et al. 1986; Gilbert and Goldzieher, 1946). One possible reason for the limited documentation is the difficulty in identifying that an acute vascular event, such as a myocardial infarction, has been precipitated by acute hypoglycaemia. The sympatho-adrenal response resulting from acute hypoglycaemia *per se* plus the stress of the acute vascular event may provoke a rise in blood glucose and so mask antecedent hypoglycaemia. The development of hypoglycaemia preceding and provoking an acute vascular event may often be unrecognised and

underestimated. However, hypoglycaemia promotes a major increase in myocardial contractility, heart rate and cardiac output, plus a widening of pulse pressure (Odeh et al. 1990), and this may compromise the coronary circulation in a patient who has coronary heart disease. Hypoglycaemia can also provoke cardiac arrhythmias.

Table. 1.1.4

PROARRHYTHMIC EFFECTS OF HYPOGLYCAEMIA
increased plasma adrenaline concentration
increased sympathetic drive
hypokalaemia
sympathovagal imbalance

The possible mechanisms promoting cardiac arrhythmias are indicated in Table 1.1.4. Increased ectopic activity of atrial, nodal and ventricular origin in type 1 diabetic (Fisher and Frier, 1993), and non-diabetic (Leak and Starr, 1962) individuals, with (Leak and Starr, 1962), and without (Fisher and Frier, 1993) evidence of coronary heart disease, has been documented. Spontaneous reversion to sinus rhythm is usual following correction of hypoglycaemia.

Hypoglycaemia is associated with specific ECG changes which include flattening or inversion of the T wave (Lloyd-Mostyn and Oram, 1975), QT prolongation (Egali and Berkmen, 1960) and ST segment depression (Lloyd-Mostyn and Oram, 1975), although the latter effect may reflect co-existing myocardial ischaemia. The T wave changes can be reversed by simultaneous infusion of potassium (Egali and Berkmen, 1960), and are absent during concurrent beta-blockade (Lloyd-Mostyn and Oram, 1975). Hypoglycaemia-induced cardiac arrhythmia has been proposed to be a precipitant of the 'Dead in Bed' syndrome, a phenomenon describing the sudden

death of healthy young type 1 diabetic patients during sleep (Tattersall and Gill, 1991).

Renal system

Renal haemodynamics are affected by hypoglycaemia with a fall in glomerular filtration rate and total renal blood flow (Patrick et al. 1989), and the changes are more pronounced in subjects with type 1 diabetes (Patrick et al. 1992). Secretion of plasma catecholamines and activation of the renin-angiotensin system are thought to be implicated. In diabetic patients who have established nephropathy, these changes in glomerular haemodynamics and rate of filtration plus other attendant effects of hypoglycaemia might promote further deterioration in renal function, but this is purely speculative.

Gastro-intestinal system

Hypoglycaemia causes transient mild hepatic dysfunction with subtle changes in some liver enzymes that are probably of no clinical significance (Howie et al. 1989). Hypoglycaemia does not appear to influence glucose absorption from the gut in healthy non-diabetic subjects (Moller et al. 1992) but controversy surrounds the effect on gastric emptying during hypoglycaemia. Gastric emptying has been shown to be accelerated in patients with type I diabetes (Schvarcz et al. 1993) but hypoglycaemia did not effect pyloric activity in non-diabetic humans (Fraser et al. 1991). It is not clear whether these changes have clinical importance in diabetic patients and contribute to the morbidity of this state. Patients with autonomic neuropathy may develop hypoglycaemia as a result of gastric stasis delaying subsequent absorption of glucose from the small bowel.

The Eye

Acute hypoglycaemia causes a sudden fall in intraocular pressure (Frier et al. 1987). The acute changes in intra-ocular pressure will increase perfusion pressure and capillary flow in retinal vessels and it has been postulated that such acute haemodynamic changes may precipitate capillary closure, or even the rupture of fragile new vessels causing haemorrhage (Frier and Hilsted, 1985). It was also suggested that the acute effects on haemostasis and haemorrheology induced by hypoglycaemia, coupled with haemodynamic changes in various vascular beds, may effect blood flow in the microvasculature of diabetic patients who have established microangiopathy (Frier and Hilsted, 1985). The changes in capillary blood flow and the increased blood coagulability could promote capillary closure, with the exacerbation of established background retinopathy and progression of diabetic microangiopathy in other tissues. Circumstantial evidence to support this hypothesis is provided by the Kroc, Steno and Oslo studies (Lauritzen et al. 1983; Dahl-Jorgenson et al. 1985; Hanssen et al. 1986), which showed an initial deterioration in diabetic retinopathy with the establishment of strict glycaemic control. A similar phenomenon was observed in the DCCT in type 1 diabetic patients' exposed to strict glycaemic control (The Diabetes Control and Complications Trial Research Group, 1993). Hypoglycaemia does not cause microangiopathy, but the pathophysiological changes that it promotes may have deleterious effects on a diseased microvasculature.

Central Nervous System

The clinical manifestations of hypoglycaemia are protean. Variable losses of sensory and/or motor functions and convulsions have been reported. Features of decortication or decerebration (Seibert, 1985) may occur, and focal abnormalities are well described such as hemiplegia (Foster and Hart, 1987; Lala et al. 1989),

choreoathetosis (Newman and Kindel, 1984), ataxia (Ingram et al. 1967), and severe amnesia (Chalmers et al. 1991). In individual case reports structural abnormalities have been observed by nuclear magnetic resonance imaging following hypoglycaemia although CT scanning was normal (Chalmers et al. 1991; Perros et al. 1994). A recent case was reported of a type 1 diabetic patient who became hypoglycaemic during a contrast enhanced CT scan. The CT scan revealed a non-enhancing low density area in the left internal capsule that disappeared after treatment with glucose (Koppel and Daras, 1993). Intermittent hypoglycaemia may not be diagnosed in elderly diabetic patients who are often assumed to be suffering from transient ischaemic attacks. In general many of these episodes of neurological dysfunction are reversed by the administration of glucose and permanent neurological abnormalities are rare.

The pathophysiology of many transient episodes of neurological dysfunction remains unclear. It is not unusual to find localised premature cerebrovascular atherosclerosis in diabetic patients which might contribute to transient neurological dysfunction or cerebrovascular infarction induced by hypoglycaemia. However, this explanation is unlikely in children, or in adults who experience transient paralysis on alternating sides (Foster and Hart, 1987). An acute change in regional cerebral blood flow (CBF) causing local ischaemia is a more likely cause of this transient neurological dysfunction.

Convulsions are a well-recognised sequel of acute hypoglycaemia, occurring often in type 1 diabetic children. Idiopathic epilepsy is over-diagnosed in adult type 1 diabetic patients with convulsions and the prevalence of epilepsy is considered to be similar to that of the non-diabetic population. One diagnostic problem is the difficulty in interpreting the electroencephalogram (EEG) following a hypoglycaemic convulsion because abnormalities may occur with hypoglycaemia per se and can persist for up to several days. In addition a significant proportion of

diabetic patients who have experienced severe recurrent hypoglycaemia develop permanent EEG abnormalities (Gold et al. 1993).

Physiology of hypoglycaemia

Hypoglycaemia rarely occurs in a non-diabetic population. In non-diabetic humans the ambient blood glucose concentration is tightly controlled by homeostatic mechanisms. Because of the vital importance of glucose to the brain, normoglycaemia is maintained by several counterregulatory systems, without which an individual would become progressively hypoglycaemic even in the absence of hyperinsulinaemia. The counterregulatory hormones have been shown to have differing abilities to promote recovery from hypoglycaemia, with the most potent being glucagon and adrenaline. Some hormones such as adrenaline and vasopressin, which are released in response to hypoglycaemia, have significant haemodynamic effects that influence vascular flow and perfusion of tissues, and promote haemostatic changes effecting intravascular coagulability. In the following section I will review our current understanding of the physiology of glucose counterregulation during insulin-induced hypoglycaemia.

Hormonal counterregulation

The kinetics of glucose recovery from insulin-induced hypoglycaemia is such that factors other than simple insulin dissipation must be involved. Acute glucose production is predominantly secondary to hepatic glycogenolysis although gluconeogenesis is initiated very early on during hypoglycaemia (Garber et al. 1976). The onset of glucose recovery is also marked by a considerable reduction in peripheral glucose utilisation (Garber et al. 1976). As this occurs in the presence of plasma insulin levels that are considerably higher (100-fold in Garber et al. 1976) than baseline levels then additional factors must be involved in glucose

counterregulation. Glucose counterregulation has now been extensively investigated and we are beginning to understand its physiology. The important glucose counterregulatory factors include hormones, neurotransmitters and metabolic substrates/intermediates.

The most important counterregulatory hormone is glucagon, which is secreted by the pancreatic islet α cells. It stimulates hepatic glucose production, raising blood glucose levels within minutes, through glycogenolysis and gluconeogenesis (Cryer, 1993b). Adrenaline, released from the medullary region of the adrenal gland, is the second most important counterregulatory hormone, and like glucagon, has a very rapid effect on blood glucose levels (Cryer, 1993b). Its action is more complex, but essentially stimulates hepatic glucose production and limits peripheral glucose utilisation. Because adrenaline stimulates hepatic gluconeogenesis through mobilisation of peripheral precursors (lactate, alanine) to the liver (Frizzell et al. 1988), and stimulation of lipolysis and ketogenesis (Fanelli et al. 1993), its hyperglycaemic effect is more sustained. Adrenocorticotrophin (ACTH) and growth hormone (GH) are also important glucose counterregulatory hormones, but their effects only become apparent after several hours. GH and cortisol (ACTH-stimulated) predominantly limit glucose utilisation but also stimulate lipolysis (Rizza et al. 1982b; Rizza et al. 1982a).

The two main neurotransmitters that have been shown to effect glucose production during hypoglycaemia are noradrenaline and acetylcholine. Noradrenaline has a similar action to adrenaline (Clutter et al. 1988), whereas acetylcholine inhibits hepatic glucose production (Boyle et al. 1988a). It was previously felt that the concomitant secretion of glucagon, which has the opposite effect, masked the inhibitory effect of acetylcholine on hepatic glucose production. Should a defect in glucagon secretion then develop in a type 1 diabetic subject the acetylcholine action might render them more susceptible to hypoglycaemia. In fact, rates of glucose

production and utilisation are unaltered during insulin-induced hypoglycaemia in glucagon deficient type 1 diabetic subjects who have been given a muscarinic antagonist (Hvidberg et al. 1996), which would suggest the acetyl-choline effect is of no great significance in glucose counterregulation.

Other metabolic fuels are also thought to contribute to the hypoglycaemic response. Plasma lactate levels rise during hypoglycaemia and reflect increased muscle glycogenolysis (Davis et al. 1997). This is thought to be a reflection of adrenaline action on muscle glycogenolysis, although more recent data suggest noradrenaline may be as important (Maggs et al. 1997). Lactate is a substantive precursor for gluconeogenesis and can potentially be used acutely by the brain (Veneman et al. 1994) [the use of alternative fuels for brain metabolism will be discussed later]. Lipolysis is also an important part of the metabolic response to hypoglycaemia (Frizzell et al. 1988; Davis et al. 1997). Glycerol is a substrate for gluconeogenesis and increased non-esterified fatty acids (NEFA) in the periphery limit glucose utilisation owing to substrate competition. Furthermore, the liver converts NEFA to ketone bodies, which could be used by the brain as an alternative fuel (Davis et al. 1993a). The effects of hypoglycaemia on peripheral metabolism were originally thought to reflect adrenaline release from the adrenal medulla. This is because of the known effects of adrenaline on peripheral metabolism and because during hypoglycaemia adrenaline comprises about 85% of the catecholamine response (Maggs et al. 1997). However, a recent study that examined adrenaline, noradrenaline and metabolic substrates in the ECF using microdialysis catheters has suggested that noradrenaline, released locally from the neuroeffector junction, comprises about 35% of the catecholamines in adipose tissue and 50% in skeletal muscle i.e. local noradrenaline release was as important in determining the peripheral response to hypoglycaemia (Maggs et al. 1997). This study also shed some light on local metabolic changes during hypoglycaemia. While it was confirmed that peripheral tissues (especially muscle) released potentially important

gluconeogenic precursors, it was shown that ECF glucose actually showed a greater relative fall during hypoglycaemia implying an increased efficiency of glucose extraction during hypoglycaemia in skeletal muscle and adipose tissue i.e. local glucose transport has adapted acutely to hypoglycaemia.

It is currently believed that there is a redundancy amongst the glucose counterregulatory factors and a hierarchy in their relative importance (Cryer et al. 1994; Cryer, 1993b). This subject has been reviewed extensively (e.g. Cryer et al. 1994; Cryer, 1993b), and will be briefly summarised here.

Hypoglycaemia induces the release of many different counterregulatory hormones, neurotransmitters and metabolic substrates. However, only a few of these appear to have physiological relevance to hypoglycaemia recovery. In a series of studies over the last 20 years it has been shown that glucagon plays a primary role in glucose counterregulation, that adrenaline is not normally crucial but becomes so in the absence of glucagon, that growth hormone and cortisol are important in continued but not acute glucose counterregulation, and that other hormones, neurotransmitters, and metabolic substrates probably do not play an essential role in the acute response to hypoglycaemia (Cryer, 1993b). This was demonstrated most clearly in the work by Gerich et al. (1979), where glucose recovery to insulin-induced hypoglycaemia did not occur in somatostatin infused (blocking glucagon release), bilaterally adrenalectomized human subjects.

The threshold for release of the glucose counterregulatory hormones during insulin-induced hypoglycaemia has also been extensively studied. The definitive study was probably that of Mitrakou et al. (1991), who used a stepped hyperinsulinaemic glucose clamp technique with measures of arterialised venous blood and established the threshold for glucagon and adrenaline release to be 3.0 mmol/l. More recent studies (see later) have indicated that the threshold is in fact a very plastic

phenomenon and many factors can influence its absolute value.

Impaired hormonal counterregulation

The developments in our understanding of the physiology of glucose counterregulation have provided essential information to explain the occurrence of hypoglycaemia in the insulin-treated population and to explain the particular propensity of some individuals to hypoglycaemia. Whilst it is clear that insulin, if present in sufficient quantities, will always overcome the body's natural defence mechanisms it is recognised that some individuals are particularly prone to hypoglycaemia.

A deficient glucagon response to insulin-induced hypoglycaemia is common in type 1 diabetic individuals and develops as an acquired defect within the first few years after diagnosis (Bolli et al. 1983). It is a selective defect in that glucagon responses to other stimuli remains intact. The cause is unknown but its relationship to absolute insulin deficiency suggests an influence of β -cell loss. Similarly, many patients with relatively long-standing type 1 diabetes (>10 years) also have a deficiency in the adrenaline secretory response to insulin-induced hypoglycaemia (Bolli et al. 1983). As with the glucagon abnormality the deficiency is selective, and of unknown mechanism.

Patients with combined deficiencies in glucagon and adrenaline responses to hypoglycaemia have the syndrome of defective glucose counterregulation (Cryer, 1993b). Such patients, when compared to type 1 diabetic subjects who have an intact adrenaline response to hypoglycaemia, have a 25-fold increased risk of severe hypoglycaemia (White et al. 1983; Bolli et al. 1984).

Other factors effecting the counterregulatory hormonal response to hypoglycaemia

Single or repeated episodes of antecedent hypoglycaemia cause elevated glycaemic thresholds (lower plasma glucose concentrations) for several counterregulatory responses (particularly adrenaline) to subsequent hypoglycaemia. This finding has been confirmed in insulinoma patients (Mitrakou et al. 1993), in healthy non-diabetic individuals (Heller and Cryer, 1991; Davis and Shamoon, 1991; Widom and Simonson, 1992; Veneman et al. 1993; Davis and Shamoon, 1991) and type 1 diabetic subjects (Davis et al. 1992; Dagogo-Jack and Cryer, 1993; Kedes and Field, 1964; Lingenfelser et al. 1993b; George et al. 1997). Most studies have been either of rather short duration (24hrs) or of much longer duration so that it is difficult to say how long this defect persists. George et al. (1995) demonstrated in non-diabetic individuals that an impaired counterregulatory hormone response to subsequent hypoglycaemia could be detected 5 days after the initial episode of experimental hypoglycaemia, whilst others (Robinson et al. 1995) have found that the counterregulatory defect persisted for more than 6 days but less than 28 days. However, when George et al. 1997 studied type 1 diabetic individuals (using the same protocol as in their previous study) the counterregulatory defect was found to have returned to baseline between 1 and 2 days from the initial episode of experimental hypoglycaemia. It is possible that the competing influences of alternating hypo- and hyperglycaemia in some way compensate for each other, and the effect of antecedent hypoglycaemia on subsequent hypoglycaemia may be less sustained in the type 1 diabetic population.

Different degrees of antecedent hypoglycaemia have been shown to have a differential effect on subsequent hypoglycaemia. A recent study (Davis et al. 1997) examined the effect of 4 differing glycaemic states on a standardised day 2 hypoglycaemic stimulus. Mild day 1 hypoglycaemia (3.9 mmol/l) was found to significantly blunt subsequent adrenaline (~40%) and glucagon (~40%) responses

compared with day 1 euglycaemia. A slightly more pronounced day 1 hypoglycaemia (3.3 mmol/l) had the same effect on adrenaline and glucagon as mild day 1 hypoglycaemia but also effected changes in growth hormone, noradrenaline and pancreatic polypeptide. Finally, there was no further effect of day 1 moderate hypoglycaemia (2.9 mmol/l) on subsequent responses to that noted when day 1 hypoglycaemia was 3.3 mmol/l. This study showed that, up to a maximum of ~3.3 mmol/l, the more intense the hypoglycaemic episode the greater its suppressive effect on the counterregulatory response to subsequent hypoglycaemia.

Some explanation for the effects of antecedent hypoglycaemia on subsequent hypoglycaemia can be gleaned from two elegant experiments reported by Davis et al. (1994, 1997). In the first of these experiments it was shown that in normal humans a physiological increase in cortisol, mimicking the levels that can be found following acute hypoglycaemia, could produce a similar counterregulatory hormonal deficiency during subsequent hypoglycaemia as could an episode of antecedent hypoglycaemia (Davis et al. 1994). Subsequently, it was shown that if cortisol was maintained at physiological levels (this was done by using a continuous subcutaneous infusion of hydrocortisone) in a group of patients with Addison's disease, then the effects of a single episode of hypoglycaemia on hypoglycaemia induced the following morning were ameliorated (Davis et al. 1997). Both these experiments would suggest that the increase in cortisol following an episode of hypoglycaemia is responsible for the impaired counterregulatory response to subsequent hypoglycaemia.

It has been argued that if antecedent hypoglycaemia causes a defect in the counterregulatory responses to subsequent hypoglycaemia then it follows that strictly avoiding hypoglycaemia could restore the thresholds for stimulation of the counterregulatory response to normal. This, it is argued, should not entail a change in overall glycaemic control. Certainly there are a few small studies that have

suggested that strict avoidance of hypoglycaemia does indeed reverse the counterregulatory hormonal defect (Fanelli et al. 1993; Fanelli et al. 1994; Cranston et al. 1994).

The first evidence that the counterregulatory defect associated with antecedent hypoglycaemia could be reversed came from a study in an insulinoma patient (Maran et al. 1992). By studying this subject using a stepped glucose clamp the authors were able to show that post-operatively, the magnitude of the counterregulatory response to experimental hypoglycaemia increased significantly and that the threshold for the adrenaline response to hypoglycaemia increased from a plasma glucose of 1.9 mmol/ to 2.7 mmol/l (Maran et al. 1992) (N.B. this was not 'normalised'). A similar finding was reported by Vea et al. (1992) in another insulinoma patient. Finally, Mitrakou et al. (1993) performed stepped hypoglycaemic clamps in 6 insulinoma patients preoperatively and 6 months post-operatively. This group found, in comparison with 14 normal subjects examined at the same time points, that pre-operatively the insulinoma patients had an impaired symptomatic and counterregulatory responses to hypoglycaemia, but that post-operatively there were no significant differences between groups (i.e. surgery had reversed the abnormalities, although it is notable that the thresholds for autonomic and neuroglycopenic symptoms and for each of the counterregulatory hormones were higher than those of the non-diabetic individuals in all cases). An insulinoma patient probably experiences what is effectively chronic hypoglycaemia and therefore the interpretability of these findings to type 1 diabetic patients, who experience alternating hyper- and hypoglycaemia, is in some doubt.

Fanelli et al. (1993) reported that the meticulous prevention of hypoglycaemia over a 3 month period in intensively treated type 1 diabetic subjects of short duration of disease improved, though did not normalise, the magnitude and glycaemic thresholds of the counterregulatory hormonal, symptom and cognitive response to

experimental hypoglycaemia. Similarly, Cranston et al. (1994) performed stepped hypoglycaemic clamps on 6 type 1 diabetic subjects with near-normal glycaemic control (mean HbA1c=6.5%) and 6 type 1 diabetic subjects with moderate glycaemic control (mean HbA1c=8.2%), who had experienced frequent hypoglycaemia before and after 3 weeks of strict absence of hypoglycaemia. This group found that avoidance of hypoglycaemia lead to a significant improvement in the magnitude of the counterregulatory response to hypoglycaemia and lowered the thresholds at which the hormonal and symptom responses were stimulated. However, although reported as not significant, HbA1c did increase in both study groups (by ~0.5%) and it is therefore possible that prevailing glycaemic control has influenced the outcome measures and that a type II statistical error is being reported. In a further study of this nature (n=6 type 1 diabetic subjects with normal awareness; n= 6 type 1 diabetic subjects with unawareness; n=6 non-diabetic controls) it has been reported that strict avoidance of hypoglycaemia over 3 months lead to an improvement in the threshold for symptomatic awareness of hypoglycaemia but had no effect on the thresholds for the hormonal response to hypoglycaemia (Fanelli et al. 1994). Finally, Liu et al. (1996) reported that following 3 months of less strict glycaemic control (mean HbA1c rising from 6.9 to 8.0%) there was a general improvement in the thresholds for the hormonal and symptomatic responses to hypoglycaemia in 7 type 1 diabetic subjects with strict glycaemic control and unawareness.

All of these studies were very labour intensive and unlikely to influence routine clinical practice at this stage. It is clear that hypoglycaemia *per se* can disrupt the counterregulatory and symptomatic response to subsequent hypoglycaemia, and that this effect probably persists for between 24 and 48 hrs in type 1 diabetic subjects. For those subjects prone to recurrent hypoglycaemia or with hypoglycaemia unawareness, a short period of hypoglycaemia avoidance with some relaxation of glycaemic control may partially restore some of these defects.

The role of insulin in modulating the counterregulatory response to hypoglycaemia is debated. Diamond et al. (1991) showed that raising insulin levels 10-fold suppressed the magnitude of the counterregulatory response to hypoglycaemia. Kerr et al. (1991) found that increasing insulin levels 3-fold also suppressed some of the components of the counterregulatory response to hypoglycaemia (growth hormone but not adrenaline). In neither of these studies did the threshold for triggering the response change significantly. Davis et al. (1992), similarly studied the effect of insulin on counterregulation, but found that when insulin concentrations were increased 6- to 8- fold there was an increase in the adrenaline, noradrenaline and cortisol responses to hypoglycaemia. This finding was shown in animal studies initially (Davis et al. 1992), and then replicated in normal volunteers (Davis et al. 1993a), but was not confirmed in type 1 diabetic subjects (Davis et al. 1993b). The pharmacological doses of insulin used and the small numbers in each study group to a certain degree compounded all of these studies. In a much larger study Lingenfelser et al. (1996) assessed the counterregulatory, neurophysiological and symptomatic responses to equivalent hypoglycaemia under two different but more physiological hyperinsulinaemic glucose clamp experiments in 27 type 1 diabetic subjects. This group showed that a higher degree of physiological hyperinsulinaemia caused an enhanced counterregulatory (adrenaline, noradrenaline, growth hormone, cortisol), symptomatic (autonomic and neuroglycopenic) and neurophysiological (auditory evoked potentials) to an equivalent degree of hypoglycaemia. All these studies seem to suggest that insulin can modulate the physiological responses to hypoglycaemia, but how this happens is unknown.

Other factors that have been shown to influence the magnitude and threshold of the counterregulatory response to hypoglycaemia include age (Meneilly et al. 1995; Amiel et al. 1987a) and sex (Draeos et al. 1995). The effect of autonomic dysfunction is debated but in general has not been shown to influence responses to

hypoglycaemia (Hepburn et al. 1990).

The influence of alternative fuels on glucose counterregulation.

It is recognised that there are alternative substrates in the brain that it could potentially oxidize, and which might therefore have a physiological action in limiting the degree of brain dysfunction during hypoglycaemia. Amiel et al. (1991) argued that the rebound increase in ketones which occurs during hypoglycaemia could provide an alternative substrate for the brain. During prolonged fasting the brain is able to use ketones as a fuel substrate (Owen et al. 1967). However, during acute insulin-induced hypoglycaemia the issue is more debated. Ketone infusion has either been found to have no effect on hormonal counterregulation in response to acute insulin-induced hypoglycaemia (Frolund et al. 1980), or there has been evidence of brain utilisation of ketones during acute hypoglycaemia (Amiel et al. 1991a; Veneman et al. 1994). In the later two studies (Amiel et al. 1991a; Veneman et al. 1994) β -hydroxybutyrate was infused prior to the induction of hypoglycaemia, however, if the rise in ketones following hypoglycaemia is prevented no effects on brain function can be detected (Fanelli et al. 1993). Altogether, this suggests that although the brain does appear to be able to utilise ketones as a fuel in the normal day-to-day context of acute hypoglycaemia this is unlikely to have a significant impact on hormonal counterregulation to hypoglycaemia.

Similarly, it has been postulated that lactate could serve as an alternative fuel substrate. Animal studies have indicated an increased uptake of lactate by the brain during hypoglycaemia (Avogaro et al. 1990). Furthermore, during strenuous exercise in humans (where plasma lactate can rise as much as 10-fold), plasma glucose levels can decrease to <3.0 mmol/l without any apparent symptoms of hypoglycaemia (Felig et al. 1998). In a small group of 10 non-diabetic subjects the infusion of lactate just prior to the onset of hypoglycaemia raised the threshold for the

counterregulatory response to hypoglycaemia and reduced the magnitude of the response (by as much as 73% in the case of adrenaline) (Veneman et al. 1994). Furthermore, the post-hypoglycaemia infusion of lactate reduces the development of symptoms acutely, reduces counterregulatory responses and prevents further deterioration of cognitive function to continuing hypoglycaemia (Maran et al. 1996). However, in these studies pharmacological concentrations of lactate have been used. The recognised increase in lactate during hypoglycaemia is substantially less and although an important aspect of the metabolic response to acute hypoglycaemia (preventing prolonged hypoglycaemia) its role in effecting thresholds for hormone release is unclear.

Localisation of the 'glucosensor'

In order for a physiological response to hypoglycaemia to be generated, the body needs to be able to detect a low blood glucose and co-ordinate a response designed to protect it from hypoglycaemia. A role for both CNS and extra-cerebral glucose 'sensors' has been proposed (Biggers et al. 1989; Donovan et al. 1991). Although a role for glucose counterregulation in the liver (Donovan et al. 1991) has been proposed, the bulk of evidence points to the CNS as the dominant centre for the sensing and integration of hypoglycaemia signals. A bilateral infusion of glucose into both carotid and vertebral arteries, designed to maintain cerebral euglycaemia in the face of systemic hypoglycaemia, nearly abolishes hormone release during hypoglycaemia (Biggers et al. 1989; Frizzel et al. 1993). Whilst these studies provide evidence of the key role played by the brain in hormonal counterregulation they do not localise the putative 'glucosensor' to any specific part of the brain.

More recently, information has emerged that the ventromedial hypothalamus (VMH) is essential for hypoglycaemic counterregulation. The glucagon and catecholamine responses to hypoglycaemia are markedly attenuated in rats with bilateral VMH

lesions, compared with rats with lesions in other parts of the brain (Borg et al. 1994). It has also been shown that if localised glucopenia is established in the VMH there is a prompt increase in counterregulatory hormones (Borg et al. 1995). Borg et al. (1997) recently showed in chronically catheterised rats that perfusion of the VMH with either L-glucose (a non-metabolizable form of glucose) or an iso-osmotic solution lacking glucose resulted in prompt and marked increases in the concentrations of glucagon and catecholamines. In contrast, in the face of systemic hypoglycaemia, the local perfusion into the VMH of D-glucose inhibited the counterregulatory response. Moreover, the counterregulatory response was only partially attenuated by infusing 15 mmol D-glucose, but 85% suppressed when 100 mmol D-glucose was infused. This suggests that the VMH contains gluco-receptors that sense fluctuations in glucose concentrations and modulate the counterregulatory response to hypoglycaemia, perhaps through the release of neurotransmitters.

In support of this hypothesis, it has been reported that insulin-induced hypoglycaemia stimulates noradrenaline release in the VMH (Shimizu and Bray, 1990). Furthermore, alterations to K_{ATP} channels have been shown to affect a change in γ -aminobutyric acid (GABA) release (During et al. 1995). In a previous study by During et al. (1995) microdialysis probes were placed in the substantia nigra of the rat, a region of the brain rich in K_{ATP} channels. Perfusion with 10mM glucose increased GABA release two-fold, as did perfusion with the sulphonylurea, Glipizide. Perfusion with the specific K_{ATP} channel activator, lemkalim, or 2-deoxyglucose with oligomycin, inhibited GABA release. When systemic hypoglycaemia was induced nigral dialysate GABA concentrations decreased by 50% as well. This would indicate that glucose might modulate GABA release through an effect on K_{ATP} channels in a manner similar to the pancreatic beta-cell. The cellular mechanisms used by the VMH to sense and transduce the glucose signal are unknown. It is possible that the VMH may share common mechanisms for glucose-sensing with the pancreatic beta cell, which is the only glucose-sensing cell

that has been well characterised. In keeping with this possibility, cells within the medial hypothalamus have been shown to express the gene for glucokinase as well as GLUT-2 (Jetton et al. 1997). Moreover, VMH neurones contain K_{ATP} channels, which are important in signal transduction (Zini et al. 1991). Taken together, these data suggest that the VMH is integral to hypoglycaemia counterregulation, and that cells within the VMH may have the ability to 'sense' prevailing glucose levels, thus affecting a change in local neurotransmission.

Other relevant physiological changes that occur during acute hypoglycaemia

(i) Cerebral blood flow

It is recognised that the brain is normally reliant on a continuous supply of glucose from the peripheral circulation. Glucose extraction from the cerebral circulation is directly related to the surface area, as well as blood flow, and to the activity and quantity of glucose transporters. It would seem logical that cerebral blood flow (CBF) should increase before the blood glucose threshold for cognitive impairment is reached. Most studies in humans do suggest that acute hypoglycaemia causes an increase in cerebral blood flow (Tallroth et al. 1992; Tallroth et al. 1993), although some controversy remains (Powers et al. 1993). However, the increase in CBF has only been documented at very low blood glucose levels (<2.0 mmol/l), well below the threshold for cognitive dysfunction (Tallroth et al. 1993). It is possible that CBF is increased as consequence of capillary recruitment, rather than through an increase in linear flow, which would substantially increase the area for glucose transport. Recently there has been speculation that this may be responsible for the increased glucose extraction that follows prolonged hypoglycaemia (Boyle et al. 1994). Alternatively, there may simply be an increase in the number and activity of glucose transporters, although this seems to be an effect of chronic as opposed to acute changes in blood glucose (McCall et al. 1986; Kumagai et al. 1995). A recent study

(Thomas et al. 1997) demonstrated that increasing CBF by about 30% during acute hypoglycaemia (using a bolus of acetazolamide) attenuated the hormonal response to, and perception of hypoglycaemia without altering the normal cognitive responses to acute blood glucose lowering. This indicates that the brain may be differentially sensitive to increased substrate delivery. Further support for this has recently been demonstrated when it was shown in 4 non-diabetic subjects that despite an increase in regional CBF (somatosensory cortex) during hypoglycaemia the regional cerebral metabolic rate (measured during performance on a vibrio-tactile task) did not proportionally increase (Powers et al. 1996) i.e. the matched increase in regional CBF and regional cerebral metabolic rate that is seen during such task performance in non-diabetic subjects during normoglycaemia did not occur. It has also been shown that a caffeine-induced reduction in CBF enhances both the symptomatic and hormonal responses to acute hypoglycaemia (Debrah et al. 1996).

Abnormal CBF and cerebro-vascular reactivity outwith experimental hypoglycaemia is recognised in type 1 diabetes. Studies in animal models have demonstrated that CBF is decreased in poorly controlled diabetic rats (Kikano et al. 1989), while in diabetic humans diminished CBF may be related to increasing duration of disease (McCall, 1992). Cerebro-vascular reactivity, with respect both to vasodilatation and vasoconstriction, is abnormal in some diabetic patients (Croughwell et al. 1990), and abnormalities may be related to duration of disease (Auer and Siesjo, 1993). These observations suggest that while CBF may increase with hypoglycaemia, abnormalities in CBF and cerebrovascular reactivity in diabetic patients may attenuate the increment in CBF, rendering the brain more vulnerable to neuroglycopenia. In diabetic patients who have been exposed previously to recurrent severe hypoglycaemia, regional CBF is increased in specific areas of the brain, including the frontal lobes (MacLeod et al. 1994). It has been postulated that these permanent changes may represent an adaptive mechanism to protect these regions from recurrent severe hypoglycaemia causing cumulative cerebral damage.

(ii) Haemodynamic changes

The haemodynamic responses to insulin-induced hypoglycaemia are secondary to activation of the autonomic nervous system and the release of hormones such as adrenaline. Significant effects can be found in blood flow within various organs including a pronounced increase in heart rate, cardiac output and myocardial contractility. Blood flow through the skin is diminished, is increased through skeletal muscle, and declines acutely in the kidney. While this is identical to the "fight or flight" response to any form of acute stress, the changes in regional blood flow may contribute directly to glucose counterregulation. Glycogenolysis in muscle provides lactate and proteolysis yields alanine. The increased blood flow through skeletal muscle promotes their transport into the circulation. Splanchnic blood flow also rises during hypoglycaemia so increasing hepatic blood flow and delivering greater quantities of these 3-carbon substrates to the liver for gluconeogenesis.

(iii) Neurochemical changes

Because of the unique dependence of the brain on glucose as the principal energy source, it is particularly vulnerable to the effects of hypoglycaemia.

Neuroglycopenic damage can occur despite maintenance of cerebral blood flow and cardiac output. During hypoglycaemia there is an excessive release of various neuropeptides and aminoacids combined with a failure of energy-dependent re-uptake mechanisms, and a disruption in calcium ion homeostasis. This produces an accumulation of excitatory aminoacids (principally aspartate or glutamate) which cause cellular damage via specific receptors, especially the N-methyl-D-aspartate receptor (McCall, 1992). This 'excitotoxic' process differs from cerebral ischaemia or anoxia in that acidosis does not develop (McCall, 1992).

The central nervous system is more vulnerable to neuroglycopenia in a rostro-caudal direction, with the cerebral cortex (especially the frontal lobes), caudate nucleus and hippocampus being most affected, while the brainstem is much more resistant to hypoglycaemia. Selective neuronal necrosis occurs following prolonged hypoglycaemia, particularly affecting layers III and V of the cerebral cortex (McCall, 1992).

PART I

CHAPTER 2

HYPOGLYCAEMIA AND COGNITIVE FUNCTION

Acute hypoglycaemia and cognitive function

Modalities of Cognitive Function effected by acute hypoglycaemia

Hypoglycaemia is a general metabolic stress that has been shown to effect different aspects of cognitive function. In one of the first studies of the effect of hypoglycaemia on a cognitive modality Flender and Lifshitz (1976) demonstrated that hypoglycaemia led to impaired fine motor co-ordination in a group of adolescent type 1 diabetic subjects. A series of influential studies in the 1980's by Holmes et al. (1983, 1984, 1986 and 1987) followed, where the hyperinsulinaemic glucose clamp technique was used to examine the effect of hypoglycaemia on different modalities of cognitive function. This group examined tasks of short-term memory, general attention, visuospatial, memory, psychomotor and academic ability tasks. During hypoglycaemia visual reaction time was slower and fewer mathematical tasks could be performed in a limited period of time, although accuracy was maintained indicating that subjects had adopted a more cautious approach. Holmes and Colleagues (1984) also reported that verbal processing was impaired during hypoglycaemia, and that the more cognitive visual and auditory reaction time tasks showed significant slowing during hypoglycaemia, whereas the simple motor finger-tapping task did not (Holmes et al. 1987; Holmes et al. 1986).

Holmes and colleagues (1984) suggested that immediate (short-term) memory may not be affected during hypoglycaemia, but this is not consistent with most other studies that have since followed (e.g. Pramming et al. 1986; Hoffman et al. 1989; Widom and Simonson, 1990; Mitrakou et al. 1991).

Following on from these studies a series of other investigators have examined different aspects of brain function during hypoglycaemia (see Table.1.2.1). In the ensuing section I will attempt to summarise the effects of hypoglycaemia on different cognitive modalities in the light of the currently available literature.

Verbal Functions: Holmes et al. (1984) have provided the most detailed examination of the effect of hypoglycaemia on verbal functions. This group examined different aspects of verbal skills in a group of type 1 diabetic individuals during hypoglycaemia. Verbal skills, specifically verbal fluency skills (Verbal Associative Fluency Test and Word Naming from the Stroop Word task), were slowed during hypoglycaemia. In the Verbal Associative Fluency test subjects are required to say as many words as they can think of beginning with a particular letter (usually the letters used are F, A and S). The subject is timed for 1 minute and a total score of acceptable responses recorded. The final score in this test is the sum of three separate trials with three different letters. Verbal fluency tasks are thought to be sensitive to general brain dysfunction, and poor scores are particularly associated with frontal lobe damage (Lezak, 1995). They provide a means of assessing how well a subject is able to organise his thinking and develop strategies of his own. Verbal fluency was also shown to be impaired by other groups at plasma glucose values ranging from 2.4 - 3.1 mmol/l (Pramming et al. 1986; Mitrakou et al. 1991; Fanelli et al. 1993; Fanelli et al. 1994; Mogan et al. 1994; Veneman and Van Haefen, 1994).

Table.1.2.1. The psychometric study of hypoglycaemia: Effects of mild to moderate hypoglycaemia on individual psychometric tests (studies are presented in chronological order).

STUDY	SUBJECTS	BLOOD GLUCOSE (MMOL/L)	TESTS IMPAIRED	TESTS NOT IMPAIRED
Holmes et al. 1983	12 T1DM	3.1	Reaction time, mathematical computations,	Supraspan, AVLT, MFFT, BVRT, NDRT
Holmes et al. 1984	12 T1DM	3.1	Stroop, verbal fluency	Word recognition, aspects of Stroop
Holmes et al. 1986	24 T1DM	3.1	CRT, SRT	Finger tapping, SRT
Pramming et al. 1986	16 T1DM	3.0 2.0	Digit span Letter cancellation, verbal fluency, TM-B, story recall, serial 7s	Many tests see at 2.0 mmol/l Finger tapping
Holmes et al. 1987	16 T1DM	3.1	auditory SRT and CRT	finger tapping, go /no go auditory RT
Heller et al. 1987	15 T1DM 10 N	3.2a 2.5a	RT RT	
Stevens et al. 1989	12 N	3.4a	computerised trail making, DSS	SRT, finger tapping, CFF, CRT
Hoffman et al. 1989	18 T1DM	2.7	pursuit rotor, TM-B	SRT, driving simulator
Widom and Simonson. 1990	17 T1DM 10 N	2.4 - 2.7a 2.3 - 2.7a 2.6 - 2.8a	letter cancellation trail making symbol digit modalities	digit span, word recall
Blackman et al. 1990	10 and 9 N	3.3a 2.6a	no decline reaction time	reaction time
Mitrakou et al. 1991	10 N	3.7 /3.1a 2.4a	no tests impaired TM-B, verbal fluency, Stroop, SRT, CRT, digit cancellation delayed verbal memory, backward digit span	TM-A, forward digit span
Wirsén et al. 1992	10 T1DM 12 N	1.8 - 2.0a	SRT, CRT, finger tapping, perceptual maze, DSS, verbal fluency, memory, digit span	Necker cube, perceptual speed
Cox et al. 1993	10 T1DM	2.6a	PASAT	finger tapping

Brierley et al. 1993	7 N (elderly) 6 N (young)	2.5a		TM-B, VRT
Hepburn et al. 1993	8 T1DM	1.6a	TM-A, TM-B	
Fanelli et al. 1993	8 T1DM 12 N	2.4a 2.8a	as in Mitrakou et al. 1991 (summed in z score)	
Fanelli et al. 1994	22 N	2.45a	as in Mitrakou et al. 1991 (summed in z score)	
Veneman et al. 1994	13 N	3.0a	as in Mitrakou et al. 1991 (summed in z score)	
Mokan et al. 1994	32 T1DM (aware) 11 T1DM (unaware)	2.7a 2.4a	as in Mitrakou et al. 1991 (summed in z score)	
Boyle et al. 1994	12 N	3.05a 2.5a	Stroop, finger tapping Immediate and delayed recall	Immediate and delayed recall
Gold et al. 1995	24 N	2.5a	PASAT, DSS, TM-B, CRT, RVIP (hits)	RVIP (false alarms), RVIP (reaction time)
Gold et al. 1995	10 T1DM (aware)	2.5a	PASAT, DSS, TM-B, RVIP (hits), RVIP (reaction times)	RVIP (misses)
Draeos et al. 1995	42 T1DM	2.2a	VRT, SRT, CRT, digit vigilance, TM-B, verbal memory, digit sequence learning, verbal fluency	

*N=non-diabetic. T1DM= type 1 diabetic DSS= Digit Symbol Substitution test. CFF = Critical Flicker Fusion Threshold. AVL T = Auditory Verbal Learning Test. MFFT = Matching Familiar Figures Test. BVRT = Benton Visual Retention Test. NDRT = Nelson Denny Reading Test. TM-A & B = Trail Making test A and B. RVIP = Rapid Visual Information Processing. PASAT = Paced Auditory Serial Addition Test. CRT = Choice Reaction Time test. SRT = Simple Reaction Time test. AN = autonomic neuropathy. a = arterialised blood glucose.

Memory functions: Most studies that have looked at memory during hypoglycaemia have examined short-term memory functions. The immediate recall of material, and the span of that recall (i.e. the amount of material that can be grasped or encoded for learning), has been the principal focus for research. One of the most commonly used tests is the Digit Span subtest of the Wechsler Memory Scale. The Digit Span test comprises two different sub-tests, Digits Forward and Digits Backward, both of which consist of seven pairs of random number sequences that the examiner reads aloud at a rate of one per second. With Digit Forward the subject's task is to repeat each sequence exactly as it is given and with Digit Backward the subject's task is to say them in reverse order. In each trial the number of digits in the sequence is increased as the subject responds correctly until the subject fails or repeats a nine-digit sequence correctly. Digit Span Forward is primarily a test of attention and can be affected by age and head injury (Lezak, 1995). Digit Span backward, which requires the brief storage of material and then some mental juggling of that material, is more of a memory test in that it is examining working memory. It is much more sensitive to diffuse brain damage such as dementia than is the Digit Forward task (Lezak, 1995). Ability on the Digit Span Forward task during hypoglycaemia has been shown to deteriorate in some (Pramming et al. 1986; Draelos et al. 1995; Wirsén et al. 1992), but not in all (Holmes et al. 1983; Widom and Simonson, 1990; Mitrakou et al. 1991) studies. Digit Span Backward was significantly impaired at a plasma glucose of 2.4 mmol/l in the work by Mitrakou et al. (1991). Both the Forward and Backward tests have also been included in the cognitive test batteries of other studies (Mokan et al. 1994; Fanelli et al. 1993; Fanelli et al. 1994; Veneman and Van Haeften, 1994). However, because z-scores were reported in these studies, it is not possible to assess the effect of hypoglycaemia on these specific tests, although z-scores in all these studies decreased significantly. Other investigators have included different memory tasks of immediate and delayed recall as part of their cognitive test battery. In general, it can be concluded from this data that the more complex the test the more likely it is to be disturbed by hypoglycaemia [e.g.

word recognition vs. verbal fluency (Holmes et al. 1984)].

Attention, Concentration and Tracking: Although these three constructs can be differentiated theoretically, in practice, it is very difficult to do so. For instance, conceptual tracking can be prevented or interrupted by a diminished ability to maintain a focused attention or concentration while solving problems. Most of the currently employed cognitive tests can be said to require sustained attention. Of the available tasks, vigilance tasks are thought to examine sustained attention in itself (Lezak 1995). Draelos et al. (1995) in a large study of 42 type 1 diabetic subjects showed a significant impairment on the Digit Vigilance task at a plasma glucose of 2.2. mmol/l. Digit vigilance has also been included in the test batteries used by others (Mitrakou et al. 1991; Mokan et al. 1994; Fanelli et al. 1993; Fanelli et al. 1994; Veneman and Van Haeften, 1994) where overall z-scores have deteriorated during moderate hypoglycaemia (plasma glucose 2.4 - 3.0 mmol/l). Other studies have also consistently shown a deterioration in sustained attention during hypoglycaemia (Widom and Simonson, 1990; Pramming et al. 1986) using different vigilance tests (e.g. letter cancellation).

The PASAT (Paced Auditory Serial Addition Test; a cognitive task that requires the subject to listen to an audiotape and perform mental additions) is another measure of attention and concentration. It is very sensitive to brain dysfunction. It is well recognised, for instance, that post-concussion patients perform well below group control averages immediately after injury but return to normal within 30-60 days. Some investigators have used the PASAT to assess cognitive function during hypoglycaemia and have shown that performance during moderate hypoglycaemia (plasma glucose 2.5 mmol/l) is significantly impaired in non-diabetic (Gold et al. 1995a) and type 1 diabetic (Gold et al. 1995b) subjects. The Digit Symbol Substitution task, and Trail Making tasks both require visuomotor tracking, attention and concentration. Both are very reliable indicators of moderate hypoglycaemia.

Because of this they are employed in many test batteries as a means of confirming the presence of cognitive dysfunction.

Executive Functions: These can be conceptualised as having four components: (1) goal formulation; (2) planning; (3) carrying out goal-directed plans; and (4) effective performance (Lezak, 1995). There are few formal tests of these abilities although most complex cognitive tasks require intact executive function and hence abnormalities in performing these tasks could reflect the disruptive action of hypoglycaemia on higher brain functions. The Stroop test is probably the most recognised test that is purported to examine executive functions. It measures the ease with which a person can change their perceptual set to changing demands (Lezak, 1995). The Stroop, or aspects of the Stroop, have been used to examine subjects during hypoglycaemia, and in general performance has deteriorated (Holmes et al. 1984; Mitrakou et al. 1991; Mogan et al. 1994; Fanelli et al. 1993; Fanelli et al. 1994; Veneman and Van Haeften, 1994). Driving performance, which clearly involves complex decision making, is disrupted by moderate hypoglycaemia (Cox et al. 1993) and will be discussed later.

Motor functions: In most studies, simple motor function like the Finger-Tapping task (principally a motor task which requires the subject to press a telegraph-type key as rapidly as possible), is not significantly effected by hypoglycaemia (Holmes et al. 1986; Stevens et al. 1989; Pramming et al. 1986; Cox et al. 1993). More complex motor tests that require a greater degree of 'executive' input, such as the Grooved Pegboard test, are disrupted by moderate hypoglycaemia (Kerr et al. 1991b). In the Grooved Pegboard test a subject is presented with a small board containing a 5x5 set of slotted holes, which are angled in different directions. The subject has to insert pegs, each of which has a ridge along one side requiring it to be rotated into position for correct insertion, into the relevant slots. Time to completion of the task is scored.

Conclusions: Some key points can be unravelled from this loose, but extensive, body of research. Firstly, cognitive tasks appear more likely to be disrupted at a given level of neuroglycopenia than are simple motor tasks. Pramming and colleagues (1986) found that moderate hypoglycaemia (2.0 mmol/l) had no effect on a simple motor task, but did disrupt more complex cognitive tasks. Similarly, in a comparative study of performance on the PASAT and on the Finger Tapping test Cox et al (1993) demonstrated that only the cognitive task was significantly disrupted during controlled moderate hypoglycaemia.

Secondly, cognitive tasks themselves also appear to show a 'hierarchy of impairment' with the more complex tasks showing abnormalities before the simpler cognitive tasks. During mild or moderate hypoglycaemia the ability to perform complex tasks that require decision making, sustained attention, and planning capacity is disrupted (Holmes et al. 1986; Stevens et al. 1989; Hoffman et al. 1989; Mitrakou et al. 1991), whereas performance of simple cognitive tasks such as digit span and simple reaction times is preserved (Stevens et al. 1989; Holmes et al. 1986; Hoffman et al. 1989; Wirsén et al. 1992). It had been shown earlier that mild hypoglycaemia did not effect the simple perceptual and cognitive task of word recognition, while complex associative word skills did show disruption (Holmes et al. 1984). Driesen and Colleagues (1991) found that only choice reaction times were prolonged during moderate hypoglycaemia (plasma glucose 2.8-3.3 mmol/l) in type 1 diabetic patients, while at lower plasma glucose concentrations (2.1 mmol/l) both simple and choice reaction times were impaired.

Finally, it has been shown repeatedly that there is considerable variability in the blood glucose level at which individuals begin to show dysfunction on any particular cognitive task. This will be discussed in more detail in a later section.

Driving requires complex decision-making and sustained attention. Its importance in everyday life makes it an ideal task to examine. Hoffman et al. (1989) did not show any significant abnormalities in driving performance in a small group of type 1 diabetic subjects at a venous blood glucose of 2.7 mmol/l. However, the type of simulator involved was thought unlikely to provide a good comparison with actual driving conditions. More recently, Cox et al. (1993) did demonstrate significant impairment of driving skills during controlled hypoglycaemia (arterialised blood glucose 2.6 mmol/l) using a more sophisticated driving simulator. Subjects showed deficiencies in steering ability, increased swerving and spinning, and poor road positioning. Another notable observation was that many patients themselves did not reliably recognise that their driving ability was affected by this moderate degree of hypoglycaemia and stated that they would continue to drive under these circumstances.

Neurophysiological changes during acute hypoglycaemia

It is now established that cognitive dysfunction occurs during acute hypoglycaemia. However, many psychometric tests require the performance of many distinct cognitive functions in concert. For example, the reaction time is the end-result of the time consumed by several cognitive processes such as selective attention and motor-related processes, even when the simple reaction time procedure is used. Because of this some investigators have suggested that neurophysiological tests provide a more 'objective' assessment of cognitive function.

The most commonly applied electrophysiological tests are evoked (EP) and event-related potentials (ERP). An evoked potential represents the neuronal response to a given stimulus (e.g. the Visual Evoked Potential (VEP) is a visual evoked response

to an external stimulus such as a shift or reversal of a checkerboard pattern; this is also known as the P100 wave) and is measured using recordings from small electrodes placed around the scalp. The placement of these electrodes has been standardised. An event-related potential is the neuronal response to an external stimulus or event, but occurs only when the subject is selectively attentive to that stimulus, and is required to distinguish one stimulus (the target) from a group of other stimuli (the nontargets). This means that the event-related stimulus is dependent on the setting of the target, and not its physical characteristics, whereas the evoked potential is very dependent on the physical characteristics of the stimulus and independent of whether the subject is attentive or interested in the stimulus.

The simplest experimental design incorporating these two potentials is the auditory 'odd-ball' task. Here a subject is presented a sequence of two distinguishable stimuli, one of which occurs frequently and the other infrequently (odd-ball). The subject is required to count mentally or otherwise respond to one of the two stimuli, and during this procedure cerebral responses to the frequent and odd-ball stimuli are recorded and averaged separately. The response to the frequent stimuli consists of a series of waves (stimulus-related components) that relates in the most part to the sensory modality studied (in this case auditory). For auditory stimuli this has been divided into three sequential periods; the early-, mid-, and long-related responses. The early related response (also known as the Brain Stem Auditory Evoked Potential; BAEP) reflects activity in peripheral and brain-stem auditory structures. The BAEP is divided into components I-V. Waves I, III, V primarily represent electrical activity for the acoustic nerve, pons, and brain stem respectively, and the latencies between them indirectly reflect neuronal conduction in the corresponding segments of the central auditory pathway. Abnormal prolongation of these latencies usually reflects a disturbance of central auditory conduction. The electrical response to the odd-ball stimulus is termed the P300 brain potential. It is a more easily identifiable wave form and, as noted, is not affected by the physical characteristics

of the stimulus. The P3, because of its sensitivity to attentional processes, is related to aspects of cognitive function. Thus, the P300 can be used to provide an index of cognitive function and the BAEP provides an index of brain-stem function.

Electroencephalographic (EEG) recordings taken during hypoglycaemia have consistently shown changes such as a slowing of alpha activity, and an increased occurrence of theta and delta waves, particularly in the frontal areas of the brain (Harrad et al. 1985; Pramming et al. 1988; Tamburrano et al. 1988; Tallroth et al. 1990; Bendtson et al. 1991). The plasma glucose levels at which these changes have been demonstrated vary from 1.5 to 2.5 mmol/l. Evoked Potentials have also been recorded during experimental hypoglycaemia (Kern et al. 1994; Tamburrano et al. 1988; Harrad et al. 1985; Ziegler et al. 1991; Jones et al. 1990). Ziegler et al (1991) observed increases in the inter-peak latencies I-III, II-IV and I-V as well as an increase in the amplitude of waves III and IV of the BAEP in a group of type 1 diabetic subjects at a mean blood glucose of 1.7 mmol/l (insulin-infusion technique) which paralleled changes in the neuroendocrine and symptomatic response to hypoglycaemia. Using a hyperinsulinaemic stepped glucose clamp, Jones et al. (1990) estimated the threshold for change in the BAEP of 8 non-diabetic subjects was 3.0 mmol/l. This was the same as the threshold for the symptomatic response to hypoglycaemia, although a higher threshold than that needed to stimulate the neuroendocrine response (3.4 mmol/l) (Jones et al. 1990). In both of these studies the 'central' waves III-IV of the BAEP seem to have been most effected by hypoglycaemia in comparison to the peripheral waveform (reflecting neural transmission in the auditory nerve). This could suggest that different neural pathways may display a variable sensitivity to hypoglycaemia. Some supportive evidence for their findings comes from studies in animals using microelectrodes placed in the inferior colliculus and which show it to be highly susceptible to hypoglycaemia (Jacob et al. 1995b; Jacob et al. 1995a; Weber et al. 1994). The neural activity in the inferior colliculus is thought to be represented by wave V of

the BAEP (Weber et al. 1994).

Hypoglycaemia has generally been found to increase the latency and decrease the amplitude of the P300 wave (Blackman et al. 1990; Blackman et al. 1992; Tallroth et al. 1990; De Feo et al. 1988; Jones et al. 1990). Blackman et al. (1990) noted that both auditory and visual P300 waves to odd-ball stimuli were significantly affected at an arterialised plasma glucose of 2.6 mmol/l, showing that hypoglycaemia was disrupting different sensory systems to a similar degree. Blackman et al. (1990) also noted in this experiment, and in a further experiment in type 1 diabetic subjects (Blackman et al. 1992), that there was a lag time of up to 75 minutes before the P300 returned to baseline after the restoration of euglycaemia indicating a delayed recovery in optimal brain functioning in the immediate period following a hypoglycaemic episode.

Whilst neurophysiological techniques for measuring brain functions are not as dependent on patient co-operation as psychometric testing, they are limited by difficulties of interpretation. The evoked potential is, for instance, very susceptible to factors such as differences in subject group and differences in electrode placement, all of which make interpretation of data difficult. The amplitude of the BAEP recorded from scalp electrodes is only 1/100th of the amplitude of the ongoing EEG activity and therefore must be extracted from this and other noise using computer averaging which, again, is susceptible to error. Furthermore, in interpreting the P300 wave it has to be accepted as axiomatic that cognitive processes are caused exclusively by physical activity in the nervous system (i.e. we assume a direct, predictable relationship between cognitive and neural processes) (Rugg and Coles, 1995). It is also assumed that a different level of activation in an event-related potential signifies a different degree or intensity of engagement of a common cognitive process. Whilst this may be a reasonable assumption it remains at present no more than an assumption. It is possible, for instance, that a change in

amplitude is the result of an effect of hypoglycaemia on the overall electrophysiological state of the brain, and may not mean that the performance of cognitive processes carried out in that state are affected. Furthermore, the P300 deflection is actually the summation of a number of smaller deflections, which are sensitive to distinct experimental factors (Rugg and Coles, 1995).

Recently, it has been reported that developments in neurophysiological research have provided methods for assessing the timing of two important cognitive processes in the brain: stimulus selection and response selection (Smid et al. 1997). Smid et al. (1997) examined 24 non-diabetic subjects during euglycaemia and hypoglycaemia (blood glucose 2.6 mmol/l), and on recovery from hypoglycaemia. Visual event-related potentials to a series of tasks utilising colour letters were measured. Hypoglycaemia was found to have a clear effect on the stimulus-selection process and an additional effect on response-selection. A differential effect was noted in the recovery period with stimulus selection returning to normal and the response-selection process remaining delayed with a continuing increase in error frequencies. The authors concluded that hypoglycaemia appeared to be exerting its main effect on executive processes (e.g. stimulus selection, stimulus-response coupling). This conclusion is perhaps a little presumptive in that the study did not actually examine more basic sensory processing and therefore could not really exclude additional effects in this aspect of information processing. Furthermore, subjects in this study were not studied twice, rather they were divided into two groups; one group undergoing the euglycaemia-placebo-euglycaemia arm the other the euglycaemia-hypoglycaemia-euglycaemia arm, and hence the study was not properly powered. It does though confirm the problems inherent in interpreting reaction time data where motor, stimulus selection and response selection processes are involved.

In conclusion, acute hypoglycaemia does lead to changes in some

neurophysiological measures and the P300 wave in particular, appears to be a sensitive index of cognitive function. However, interpretation of the neurophysiological data (especially evoked potentials) is limited by the many variables that can effect outcome measures. Also, the functional implications of an altered P300 response are unknown.

The following table details the main studies in this field, the methods used in those studies to induce hypoglycaemia and the blood glucose at which as significant change in the electrical activity of the brain was noted.

Table. 1.2.2. The Neurophysiological Study of Hypoglycaemia.

STUDY	SUBJECTS	MEASURE	TECHNIQUE	GLYCAEMIC THRESHOLD (MMOL/L)
Amiel et al. 1991	T1DM and Insulinoma	EEG	stepped clamp	1.9a
Harrad et al. 1988	Non -diabetic and T1DM	EEG /VEP (P100)	insulin-infusion	1.5-2.5v
Pramming et al. 1988	T1DM	EEG	insulin-infusion	2.0 v
Tamburrano et al. 1987	Non -diabetic	EEG /VEP (P100)	one-step clamp	2.4 (EEG only)v
Tallroth et al. 1990	T1DM and Non -diabetic	P300	insulin infusion	1.6-2.3v
Bendtsson et al. 1991	T1DM	EEG	insulin-infusion	1.4 - 1.9v
Kern et al. 1994	Non -diabetic	VEP (P100)	one-step clamp	2.7a
Jones et al. 1990	Non -diabetic	P300 /BAEP	multi-step clamp	3.0a
Ziegler et al. 1991	T1DM	BAEP	insulin-infusion	1.7 - 2.1a
Blackman et al. 1990	Non -diabetic	P300	two-step clamp	2.6a
Blackman et al. 1992	T1DM	P300	two-step clamp	2.5a
De Feo et al. 1988	Non -diabetic	P300	insulin-infusion	4.0a
Smid et al. 1997	Non -diabetic	ERP	one-step clamp	2.6a

(In this Table a =arterialised blood v =venous blood.).

Studies combining cognitive function and neurophysiological tests

Blackman and colleagues (1990) studied P300 evoked potentials and reaction times simultaneously during experimental hypoglycaemia. They found that changes in P300 evoked potentials and reaction times occurred in parallel but that the P140 wave (which measures sensory processing) was not altered by hypoglycaemia. An interesting observation from this study was that the changes in the P300 wave and reaction time occurred in parallel suggesting that some component common to both may be affected by hypoglycaemia. As the P300 wave is not a measure of motor activity this would suggest that hypoglycaemia is affecting some other component of the reaction time task such as the central integration of information within the brain (also note Smid et al. (1997); see above). Lindgren et al. (1996) found that moderate hypoglycaemia (plasma glucose 2.6 mmol/l) in 10 non-diabetic subjects affected P300 amplitudes during a relatively demanding visual search task, but not during a relatively easy task. This finding supports psychometric studies that have shown that the more complex a cognitive task the more likely it is to be disrupted by hypoglycemia.

Studies combining P300 and reaction time measures have shown consistently that, despite the blood glucose being restored to normal, a lag phase of at least 40 minutes has been observed before performance on these tasks returns to normal (Herold et al. 1985; Blackman et al. 1990; Blackman et al. 1992; Wirsén et al. 1992; Jones et al. 1990; Lindgren et al. 1996). The clinical relevance of this finding is that type 1 diabetic patients should allow sufficient time for recovery of cognitive function after an acute episode of hypoglycaemia before attempting to resume tasks such as driving or operating machinery which involve decision-making and cognitive skills.

Only a few investigators have compared type 1 diabetic subjects and matched non-diabetic controls within the same study protocol (Tallroth et al. 1990; Jones et al.

1990). In both studies basal measures of P300 latencies were significantly longer in type 1 diabetic patients, but these were not prolonged further during hypoglycaemia. However, a highly significant reduction in wave amplitude was demonstrated during hypoglycaemia in both studies. The differences between subjects with and without type 1 diabetes may have occurred because of the influence of prevailing glycaemic control. Ziegler et al (1992), reported that the threshold for changes in the latency of the P300 wave was higher for patients with strict glycaemic control (mean HbA1c 6%) than for poorly controlled patients (mean HbA1c 9%) (see section on effect of glycaemic control on cognitive function).

Blood glucose thresholds for cognitive dysfunction

It is well known that the thresholds for activation of counterregulatory hormones and symptom realisation are very plastic phenomena (Cryer, 1993a; Kimura, 1993) that can vary in the individual over relatively short time periods. They are directly influenced by such factors as antecedent hypoglycaemia (Heller and Cryer, 1991; Davis et al. 1992; Widom and Simonson, 1992; Veneman et al. 1993), or strict avoidance of hypoglycaemia (Fanelli et al. 1993; Fanelli et al. 1994; Cranston et al. 1994; Lingenfelser et al. 1995), and level of glycaemic control (Widom and Simonson, 1990; Lingenfelser et al. 1995). This subject has been extensively reviewed recently (Cryer, 1993a; Cryer, 1994).

The search for a universal threshold for cognitive dysfunction has been equally unrewarding. Variable results have been obtained which probably reflect the differing techniques used to induce hypoglycaemia, the different psychometric tests employed, and variability in individual performance. Neurophysiological studies which were thought to offer an objective means of identifying cognitive dysfunction

have similarly produced very different results (Bendtson, 1993). Holmes et al. (1983, 1984), first noted changes in cognitive function in type 1 diabetic patients at a plasma glucose of 3.3 mmol/l (using a hyperinsulinaemic glucose clamp), while Pramming et al. (1986) found changes only occurred when the venous blood glucose was less than 3.0 mmol/l (using a continuous insulin infusion). Blackman et al. (1990) and Jones et al. (1990) observed changes in p300 waves at arterialised blood glucose concentrations of 3.0 and 2.6 mmol/l respectively. More recently, using a comprehensive battery of cognitive function tests and a 'stepped' clamp technique, Mitrakou et al. (1991) observed that the initial changes in cognitive function occurred at an arterialised blood glucose concentration of 2.7 mmol/l.

While it is highly unlikely that a universal threshold for cognitive dysfunction exists, there is more controversy as to whether thresholds for an individual patient are constant. Gonder-Frederick et al. (1994) showed in a sub-group of their main study that individual performance differences were steady across time (3 months) indicating stable and characteristic responses to hypoglycaemia at least in the short-term. This result is consistent with that of Cranston et al. (1994) who noted that 3 weeks strict avoidance of hypoglycaemia altered the thresholds for counterregulatory hormone and symptom response to hypoglycaemia in a small group of patients with hypoglycaemia unawareness, but did not alter the threshold for cognitive dysfunction, although his study involved only a small number of subjects and employed only one test of cognitive function. Conflicting results are reported by Fanelli et al. (1993, 1994) who did find a change in the threshold for cognitive dysfunction following strict avoidance of hypoglycaemia, and by Boyle et al. (1994) who found that the threshold for cognitive dysfunction was altered by 56 hr interprandial hypoglycaemia. The threshold for cognitive dysfunction within an individual does not appear to be as variable as that for the neuroendocrine response to hypoglycaemia when subjected to a modifying influence such as antecedent hypoglycaemia.

Cerebral Adaptation to acute hypoglycaemia

There are a number of studies that have shown cerebral blood flow to increase during acute hypoglycaemia. It has also been demonstrated in animals that there is an increase in uptake of glucose by the brain and an increase in brain glucose transport activity during hypoglycaemia (McCall et al. 1986; Pelligrino et al. 1990; Kumagai et al. 1995). Moreover, the possibility exists that alternative fuels (e.g. lactate, aminoacids and free fatty acids) are important in brain metabolism during hypoglycaemia, although clinical studies in human subjects have not been convincing. Ketone bodies when infused prior to an episode of controlled hypoglycaemia have been shown to ameliorate the threshold and magnitude of the counterregulatory hormone response to hypoglycaemia (Amiel et al. 1991b), but not when infused concomitantly with hypoglycaemia (Fanelli et al. 1993). These effects have lead some authors to suggest that the brain may be able to adapt acutely to hypoglycaemia.

In a recent study in non-diabetic human subjects Boyle et al (1994) documented an increase in basal cerebral blood flow and a preservation of normal brain glucose uptake following prolonged (56 hr) interprandial hypoglycaemia. In this study cognitive function (measured by the Stroop test, Finger Tapping and Verbal Memory) initially deteriorated at an arterialised blood glucose level of 3.6 mmol/l, but following 56 hours of hypoglycaemia it did not deteriorate until 2.5 mmol/l. Although this study provides strong evidence for cerebral adaptation to hypoglycaemia over a relatively prolonged period, it cannot be inferred from this that the brain is able to adapt acutely to hypoglycaemia over the space of 60-90 minutes.

Kerr and colleagues (1989) examined 7 non-diabetic subjects during euglycaemia (blood glucose 4.5 mmol/l) and hypoglycaemia (blood glucose 3.5 & 3.0 mmol/l)

using the hyperinsulinaemic glucose clamp technique. Subjects were studied in a randomised single-blind manner in two experimental sessions. At the beginning and end of each glycaemic plateau the subjects performed a simple reaction time (SRT) task. The investigators found that hypoglycaemia led to a significant impairment in the SRT, but that performance returned to baseline when repeated 20 minutes later at the same blood glucose level. This, the authors suggested, indicated that cerebral adaptation to acute hypoglycaemia had occurred. In a similar study of 6 type 1 diabetic subjects, Kerr and colleagues (1991a) noted that SRT improved after 40 minutes of hypoglycaemia (blood glucose 2.8 mmol/l), in comparison to euglycaemia, and that subjects became less symptomatically aware of being hypoglycaemic. Both of these studies are limited by the small number of subjects examined, by the use of a single cognitive test (and not one that has been shown to be reliably sensitive to hypoglycaemia), and the failure to fully account for practice effects. In a larger study, designed to account for practice effects, Gold and colleagues (1995a) employed a detailed battery of cognitive function tests to examine 24 non-diabetic subjects and demonstrated that there was no evidence of improved cognitive function after 40 minutes of hypoglycaemia (plasma glucose 2.5 mmol/l).

The conclusion from the few studies that have examined this issue is that there is no good evidence to indicate that the brain is able to adapt acutely (at least within 40 minutes) in terms of brain functioning, although it does appear that a degree of adaptation occurs after 56 hours of hypoglycaemia.

Effect of glycaemic control on the threshold of cognitive dysfunction

The effect of preceding glycaemic control on the blood glucose at which cognitive dysfunction becomes evident in any individual is an area of some controversy. The question as to whether divergent changes in glycaemic thresholds for

counterregulatory responses in well controlled, intensively treated and in poorly controlled, conventionally treated type 1 diabetic subjects are mirrored by similarly directed changes in brain function is important because it is a key issue in current concepts regarding the development of hypoglycaemia unawareness, and the management strategies that have evolved to treat this condition.

There is some evidence from animal studies to suggest that the prevailing blood glucose concentration may be important in determining the threshold for a deterioration in brain functioning. In studies of diabetic and non-diabetic rats chronic hyperglycaemia and chronic hypoglycaemia, respectively, decrease and increase the efficiency of glucose extraction by the brain (McCall et al. 1982; McCall et al. 1986; Pelligrino et al. 1990). Furthermore, chronically hyperglycaemic diabetic BB rats are more susceptible, whilst chronically hypoglycaemic diabetic BB rats are more resistant to the adverse effects of hypoglycaemia on brain stem function (Jacob et al. 1995b; Jacob et al. 1995a).

In normal human subjects rendered mildly hypoglycaemic for several days and in well controlled type 1 diabetic subjects (Boyle et al. 1995), brain glucose uptake is more effectively preserved during hypoglycaemia (Boyle et al. 1994; Boyle et al. 1995). The results of neurophysiological and neuropsychological studies in human subjects have been less consistent. In the largest study to date, 42 type 1 diabetic subjects completed a detailed cognitive test battery during controlled hypoglycaemia (plasma glucose 2.2 mmol/l), euglycaemia (plasma glucose 5.5 mmol/l) or hyperglycaemia (plasma glucose 8.9, 14.4 and 21.1 mmol/l) (Draeos et al. 1995). All aspects of neuropsychological function were found to be diminished during hypoglycaemia, with some variability between tests, but no correlation was found between glycaemic control and the degree of cognitive dysfunction demonstrated. However, this study used a degree of hypoglycaemia that is considerably below the level conventionally thought to represent the threshold for cognitive dysfunction

(~2.8 - 3.2 mmol/l), and therefore, because all the tests might have been expected to change at this level, the authors were in reality comparing performance between individuals on the test themselves. This can be affected by age, sex, IQ, glycaemic control, antecedent hypoglycaemia, practice etc. With so many independent variables not accounted for, the likelihood of establishing a relationship between glycaemic control and cognitive function is small. Finally, it is not possible to calculate a threshold for cognitive change using a one-step clamp and therefore this study did not answer the question as to whether glycaemic control could alter thresholds for cognitive function

Maran and colleagues (1995) used a stepped hypoglycaemic clamp and the Four-Choice Reaction Time test to examine 8 intensively treated type 1 diabetic subjects (HbA1c $7.7 \pm 0.3\%$), 10 conventionally treated type 1 diabetic subjects (HbA1c $10.1 \pm 0.2\%$), and 8 non-diabetic subjects. They found that hormonal responses and subjective awareness of hypoglycaemia occurred at lower glucose levels in the intensively treated group, but that the threshold for a change in the reaction time test was not significantly different between groups, i.e. there was an altered hierarchy of responses to hypoglycaemia in the intensively treated group. However, in a more recent, and larger study Pampanelli and colleagues (1996) examined three groups of type 1 diabetic subjects (Group I HbA1c $<5.5\%$, Group II HbA1c $6.1-7.0\%$, Group III HbA1c $>7.5\%$) all of whom were on intensive insulin therapy. 18 non-diabetic subjects were used as controls. All subjects underwent a stepped hypoglycaemic clamp and testing using a detailed cognitive test battery. The authors found that cognitive function as expressed by the sum of z-scores, deteriorated in all groups at the lowest plasma glucose (2.3 mmol/l) but that this deterioration only reached significance in group II, group III and the non diabetic subjects. The deterioration was greater in group III as compared to group II and non-diabetics. Similar findings were noted in counterregulatory hormone and symptom responses to hypoglycaemia.

Jones et al (1997) used a multi-step glucose clamp to establish thresholds for change in the P300 wave of cortical evoked potentials in 8 intensively treated (HbA1 $8.3 \pm 0.2\%$) and 11 conventionally treated (HbA1 $14.6 \pm 1.3\%$) type 1 diabetic subjects, and 10 healthy controls. This study showed that a greater reduction in plasma glucose was required to alter P300 event related potentials in the intensively treated type 1 diabetic compared to the conventionally type 1 diabetic and non-diabetic groups. Similar results were found for counterregulatory hormone and symptom responses. These findings are supported by an earlier study by Ziegler et al (1992) who also used the P300 evoked potential but achieved hypoglycaemia by using an insulin-infusion which lowers blood glucose more rapidly. Neither of these studies adequately excluded hypoglycaemia in the few days leading up to the study days which may have influenced the outcome of their trials, and although both demonstrated a change in P300 the significance of this in terms of the actual cognitive performance of an individual remains unclear i.e. this may be having relatively little impact on brain functioning. Finally, in an earlier study using EEG changes to establish thresholds for cognitive function in a mixed group of intensively treated type 1 diabetic subjects and insulinoma patients in comparison with conventionally treated and non-diabetic controls, the exact opposite finding was reported (Amiel et al. 1991b). In this study EEG changes compatible with hypoglycaemia were evident at a blood glucose of 1.9 mmol/l in all intensively treated/insulinoma patients but in only two of the conventionally treated non-diabetic control group despite a downward shift in the blood glucose required to initiate the counterregulatory response in the intensively treated group (Amiel et al. 1991b).

It has been suggested that if thresholds for cognitive dysfunction are affected by glycaemic control then they are unlikely to be as much affected as the thresholds for other factors of the hypoglycaemic response (e.g. counterregulatory hormones). In

fact, the evidence to date does not bear this out. In those trials that have documented a change in threshold in intensively treated type 1 diabetic subjects (Jones et al. 1997; Ziegler et al. 1992; Pampanelli et al. 1996) the differences between the thresholds for cognitive and counterregulatory hormone changes have been very similar. In conclusion, it is likely that glycaemic control does have an impact on the threshold for cognitive dysfunction, however, the characteristic feature of type 1 diabetes is fluctuating glucose levels between hyper- and hypoglycaemia, and this probably diminishes the overall influence of glycaemic control on the threshold for cognitive dysfunction.

Effect of antecedent hypoglycaemia on the threshold for cognitive dysfunction

It has now been established that a short period of hypoglycaemia will impair the physiological counterregulatory hormone response to hypoglycaemia induced on the following day. The issue of whether antecedent hypoglycaemia has a similar effect on cognitive function is less clearly resolved. Veneman et al. (1993) found elevated thresholds for cognitive dysfunction after an episode of induced nocturnal hypoglycaemia in non-diabetic individuals, but Dagogo-Jack et al. (1993) found no effect of hypoglycaemia the previous afternoon on the threshold, despite demonstrated effects on the thresholds for autonomic responses and for both neuroglycopenic and autonomic symptoms, in people with type 1 diabetes. Similarly, George et al. (1997) found no effect of a single 2 hour episode of hypoglycaemia on the threshold for cognitive dysfunction during hypoglycaemia induced two days later in type 1 diabetic subjects, and Robinson et al. (1995) found no effect on day 2 and day 8 in non-diabetic subjects.

It is relevant, when asking this question, to look at the data from studies involving type 1 diabetic subjects on intensive therapy. This is because therapy that actively lowers blood glucose levels carries such a clearly increased risk of hypoglycaemia

(e.g. (Pampanelli et al. 1996)) that it is often used as a surrogate for antecedent hypoglycaemia. Elevated thresholds for counterregulatory hormone responses (Amiel et al. 1987b; Amiel et al. 1988; Amiel et al. 1991b; Clarke et al. 1991; Jones et al. 1997; Maran et al. 1995; Kinsley et al. 1995) and the symptomatic response to hypoglycaemia (Amiel et al. 1987b; Amiel et al. 1988; Amiel et al. 1991b; Clarke et al. 1991; Maran et al. 1995; Kinsley et al. 1995) have been found consistently in intensively-treated type 1 diabetic patients. If one considers the neuroglycopenic symptom response as indicative of cognitive function (i.e. that cognitive dysfunction is likely to be present if an individual is experiencing neuroglycopenic symptoms) then some (Clarke et al. 1991; Maran et al. 1995), but not all (Widom and Simonson, 1990) studies have shown elevated thresholds with intensive therapy in type 1 diabetes. However, in the study by Widom et al. (1990) a one-step hypoglycaemic clamp was used which, whilst able to show that there were statistical differences between conventionally and intensively-treated type 1 diabetic subjects in terms of their autonomic symptom and counterregulatory hormone responses to hypoglycaemia, is really unable to exclude a difference in threshold for cognitive function between the two groups. Similar discrepancies are found in the trials which have examined cognitive function using neurophysiological techniques with some (Jones et al. 1997; Ziegler et al. 1992), but not all (Amiel et al. 1988) showing an effect of tight glycaemic control on threshold for cognitive function.

Two recent studies have addressed the question more specifically (Mellman et al. 1994; Hvidberg et al. 1996). Mellman et al. (1994) studied 9 healthy volunteers using a stepped hypoglycaemic clamp technique and two cognitive tests (Logical Memory from the Wechsler Memory Scale and the Digit Symbol Substitution test from the Wechsler Intelligence Scale - Revised). Subjects underwent a 2-hour hypoglycaemic or euglycaemic clamp followed 90 minutes later by a stepped hypoglycaemic clamp. They found that antecedent hypoglycaemia had the effect of preserving ability on the Logical Memory test during ensuing hypoglycaemia (i.e.

adaptation had occurred). However, this study is unusual in that thresholds for counterregulatory hormones decreased following antecedent hypoglycaemia, which is in contradiction to all other studies in this field, and they only had cognitive data on 6 patients. Furthermore, a period of only 90 minutes separated both hypoglycaemic studies which is of very questionable relevance to the practical issues of diabetes management. In a more comprehensive study, again of non-diabetic subjects, Hvidberg et al. (1996) examined 16 young healthy volunteers using a similar study design except that the stepped hypoglycaemic clamp was performed the following morning and a more detailed cognitive test battery was used. No significant overall effect of afternoon hypoglycaemia on cognitive function during hypoglycaemia the following morning was found, although the threshold for change showed a non-significant increase on two of the tasks (Serial Addition and Delayed Non-Match to Sample tasks). The expected changes in thresholds for neuroendocrine responses to hypoglycaemia were seen.

Recently three publications have addressed the more practical issue of whether it is possible, by avoiding hypoglycaemia to normalise the threshold for the neuroendocrine response to hypoglycaemia (Fanelli et al. 1993; Fanelli et al. 1994; Cranston et al. 1994). Each study also included a cognitive test battery. Fanelli et al. (1994) found that strict avoidance of hypoglycaemia over 6 months in type 1 diabetic subjects with hypoglycaemia unawareness reduced thresholds for hypoglycaemic cognitive dysfunction (as well as those for neuroendocrine responses and symptoms), whereas, Cranston et al. (1994) reported no change for deterioration of one cognitive function test (Four-Choice Reaction Time test). It is worth noting though that in all three studies the mean HbA1c increased following avoidance of hypoglycaemia which, although reported as statistically non-significant, may have been important given the small numbers in each study. Finally, a reduction of the glycaemic threshold for cognitive function has been noted following the surgical treatment of insulinoma which effectively cured antecedent hypoglycaemia in that

study group, although this is in reality a state of chronic hypoglycaemia.

In summary, it has not been shown convincingly that antecedent hypoglycaemia significantly alters the threshold for cognitive dysfunction during hypoglycaemia. The evidence that chronic hypoglycaemia in animal, non-diabetic human, type 1 diabetic and insulinoma subjects does alter thresholds for cognitive dysfunction is clearer, but reflects an unusual physiological state. Studies of the effect of a single episode of antecedent hypoglycaemia in type 1 diabetic subjects are difficult to interpret in view of the frequent coexistence of antecedent hypoglycaemia and tight glycaemic control. It is conceivable that some cognitive processes are more sensitive than others.

Symptom change, counterregulatory hormone response, and cognitive function during acute hypoglycaemia

An area of great interest concerns the question of how the glycaemic threshold for cognitive dysfunction is related to the onset of hypoglycaemic symptoms and of counterregulatory hormonal responses. A logical answer to this question would be to suggest that as the counterregulatory response is designed to mobilise glucose stores and preferentially divert glucose to the brain, and because symptoms are a means of alerting the insulin-treated patient to hypoglycaemia, then the threshold for the counterregulatory response and onset of hypoglycaemic symptoms should be lower (a higher blood glucose) than that for cognitive dysfunction. This is borne out in those studies which have shown a rise in plasma counterregulatory hormones before any demonstrable changes in cognitive function in non-diabetic subjects (Ipp and Forster, 1987; Stevens et al. 1989; Kerr et al. 1989; Mitrakou et al. 1991; Schwartz et al. 1987). Mitrakou et al (1991) tested non-diabetic subjects and reported that elevations in plasma catecholamines and glucagon were manifest at a blood glucose concentration of 3.7 mmol/l, while autonomic symptoms occurred at

3.3 mmol/l, neuroglycopenic symptoms were experienced at 3.0 mmol/l, and significant cognitive dysfunction was not apparent until 2.4 mmol/l.

The question is more complicated when we consider the relationship between these three variables in the type 1 diabetic population. Several factors such as the quality of glycaemic control (Amiel et al. 1987b; Amiel et al. 1988; Widom and Simonson, 1990; Mookan et al. 1994; Boyle et al. 1988b; Clarke et al. 1991), antecedent hypoglycaemia (Heller and Cryer, 1991; Widom and Simonson, 1992; Davis et al. 1992), strict avoidance of hypoglycaemia (Cranston et al. 1994; Fanelli et al. 1994) the frequency of previous exposure to hypoglycaemia (Hepburn et al. 1991b; Mookan et al. 1994) duration of diabetes (Bolli et al. 1983; Mookan et al. 1994; White et al. 1983) influence the putative glycaemic thresholds for perception of symptoms and counterregulatory hormonal responses. For instance, strict avoidance of hypoglycaemia reversed the counterregulatory hormone and symptom abnormalities in type 1 diabetic patients with hypoglycaemia unawareness and long duration of disease (>10 years), but showed no effect on the threshold for cognitive dysfunction (Cranston et al. 1994). However, in unaware type 1 diabetic patients with a much shorter duration of disease (mean 5.0 years) strict avoidance of hypoglycaemia did lead to a change in threshold for cognitive dysfunction that paralleled that of counterregulatory and symptom responses to hypoglycaemia (Fanelli et al. 1994). In type 1 diabetic patients with hypoglycaemia unawareness and long duration of disease (>10 years) Dagogo-Jack et al (1994) recently reported that 3 months strict avoidance of hypoglycaemia lead to a 'normalisation' of both autonomic and neuroglycopenic symptom responses (judged by a reversal of unawareness), but no improvement in the counterregulatory response to hypoglycaemia.

Pramming et al (1986) obtained an omnibus neuropsychology score during different levels of blood glucose. A decline in overall performance was noted following the reduction in the blood glucose from 6.0 mmol/l to 3.0 mmol/l, but this did not

achieve significance until the blood glucose was reduced further to 2.0 mmol/l. Also, evidence of cognitive dysfunction was apparent in 75% of subjects prior to their becoming symptomatic of hypoglycaemia, providing evidence that a dissociation might exist between the symptomatic response to hypoglycaemia and the cognitive dysfunction that occurs.

These studies highlight the complexity of one aspect of the response of the patient with type 1 diabetes to acute hypoglycaemia. There is clearly some dissociation between the neuroendocrine, neurobehavioral and neurophysiological response to hypoglycaemia, which reflects the multiple influences that can affect each response. These factors, and more that we are as yet unaware of, contribute to the individual variability in cognitive dysfunction that is evident during hypoglycaemia.

Individual variability

Herold et al. (1985) found considerable variability in the reaction times of both type 1 diabetic and non-diabetic subjects during hypoglycaemia. Some subjects showed marked prolongation of reaction times, others had small, but significant, increases, while others showed an improvement in their reaction times. For about 50% of subjects their longest reaction time occurred up to 60 minutes after their blood glucose nadir was achieved, and for most subjects there was a delay in normalising the reaction time following restoration of normoglycaemia. This indicates a problem with sustained attention during prolonged hypoglycaemia and a delay in recovery of cognitive function following hypoglycaemia.

It is clear from many studies (Herold et al. 1985; Hoffman et al. 1989; Holmes, 1987; Cox et al. 1993; Cox et al. 1993; Gonder-Frederick et al. 1994) that the change in each individual's performance on standard cognitive performance tasks varies

greatly during hypoglycaemia. This variation is seen in both type 1 diabetic and non-diabetic subjects. Gonder-Frederick et al (1994) examined 26 type 1 diabetic subjects and found that 19 % exhibited a clinically significant deterioration in cognitive performance (which they defined as a deterioration in performance of more than two standard deviations from the baseline mean) during mild hypoglycaemia, while almost half showed little or no deterioration. When moderate hypoglycaemia was established a greater number of patients showed cognitive dysfunction (> 50 %), but 15 % still showed no evidence of cognitive dysfunction on the battery of tests used. The only correlate with poorer performance in this study was a history of previous hypoglycaemia-related unconsciousness. This study also showed that individual performance differences were stable across 3 months, indicating stable and characteristic differences in responses to hypoglycaemia at least in the short-term.

Draelos et al. (1995) found that men performed less well than women on standard cognitive tasks at a blood glucose level of 2.2 mmol/l, and also suggested that those subjects with a higher verbal IQ showed a relatively greater cognitive deterioration. More recently it has been shown that subjects with a higher score on standard measures of current intellectual ability show a significantly greater decrement in cognitive performance during hypoglycaemia in some, but not all mental tasks (Gold et al. 1995).

Type 1 diabetic patients with hypoglycaemia unawareness also appear to show a significantly greater cognitive decline during hypoglycaemia in comparison with those with retained awareness. Previously it had been shown that subjects who tended to experience neuroglycopenic, rather than autonomic, symptoms during hypoglycaemia showed a greater deterioration in reaction times (Herold et al. 1985; Hepburn et al. 1991b). A recent publication involving a group of 10 type 1 diabetic

patients with persistently impaired awareness of hypoglycaemia and a group of matched type 1 diabetic controls of the same "general cognitive ability" (as assessed by standard tests), showed a trend ($p=0.08$) towards an overall effect of awareness on cognitive function (Gold et al. 1995b). Interestingly there was a specific and significant difference between the two groups on the Rapid Visual Information Processing (RVIP) task. This implied that subjects with impaired awareness were less cautious in their responses during hypoglycaemia. During the recovery phase from hypoglycaemia those with impaired awareness performed less well on the Trail Making B task, were poorer at detecting rapid visual targets and were slower to make responses. Performance on the Digit Symbol Substitution test, Trail Making B, and the Paced Auditory Serial Addition test did not differ between the two groups during hypoglycaemia, although all were significantly disrupted. This result could suggest that impaired awareness leads to a specific, not a global, effect on cognitive ability during hypoglycaemia. From a more practical standpoint it would also suggest that type 1 diabetic patients with chronically impaired awareness show slowed reaction times *and* less accurate performance during hypoglycaemia, and that this effect can persist for up to 45 minutes, which has implications for those performing skilled tasks such as driving.

The factors so far described account for only a small amount of the variance in individual variability. (Table 1.2.3.). There is clearly a great deal more to learn about the sources of individual variability in cognitive performance during hypoglycaemia, and this information will be of great clinical relevance in educating and advising patients on insulin therapy.

Table. 1.2.3

FACTORS AFFECTING INDIVIDUAL VARIABILITY IN COGNITIVE FUNCTION DURING EXPERIMENTAL HYPOGLYCAEMIA	EFFECT ON COGNITIVE FUNCTION.
Glycaemic control	Chronic persistent hyper- or hypo-glycaemia probably do alter thresholds for cognitive dysfunction (shifted lower and higher respectively)
Antecedent hypoglycaemia	? - this issue remains unclear
Age	Elderly subjects show a relatively greater cognitive decrement
Sex	Females perform better than matched males
IQ	Higher IQ groups show relatively greater cognitive decrement
Hypoglycaemia unawareness	type 1 diabetic subjects with an impaired symptomatic response to hypoglycaemia show a relatively greater cognitive decrement
Type 1 Diabetic vs. Non-diabetic	? - this is unclear and probably relates to heterogeneity of the type 1 diabetic population in terms of such things as duration of disease, glycaemic control, daily variation in blood glucose

Chronic hypoglycaemia and cognitive function

It has been assumed that moderate episodes of acute hypoglycaemia have no permanent effect on cerebral function and that hypoglycaemia is therefore an inevitable, acceptable and benign consequence of strict glycaemic control. Evidence is accumulating however that recurrent severe hypoglycaemia may be deleterious, causing a cumulative cognitive decline with significant intellectual and psychopathological morbidity.

Studies in children

Ryan et al. (1985) used a large battery of cognitive tests to examine a group of 46 type 1 diabetic adolescents whose diabetes was diagnosed before the age of 5 years, 79 well-matched type 1 diabetic subjects who developed diabetes at an older age, and 83 non-diabetic controls. Early onset diabetes was associated with a significantly poorer performance in the following areas of mental ability: intelligence, visuospatial ability, learning and memory, attention and school achievement, and mental and motor speed. These deficits were attributed by the author to “ multiple episodes of hypoglycaemia “ (Ryan et al. 1985). Ack et al. (1961) found that children who developed diabetes before the age of 5 years were, on average, more than 10 IQ points lower than their siblings. Rovet et al. (1987,1988) found that children with onset of diabetes before 4 years of age were lower on performance IQ than sibling controls and children whose onset of diabetes was later than 4 years of age. In each of these reports severe hypoglycaemia has been identified as an important factor in the aetiology of the apparent cognitive impairment (Deary, 1993).

Golden et al. (1989) followed up 23 children with type 1 diabetes diagnosed before

the age of 5 years for a period of 6-78 months. Episodes of severe hypoglycaemia and the frequency of self-monitoring blood glucose (SMBG) measurements <2.8 mmol/l were recorded every 3 months for each individual. On completion of the trial period the children were asked to complete the Stanford-Binet Intelligence Scale. No correlation was found between any subscale of this test and the occurrence of severe hypoglycaemia. However, the relative frequency of asymptomatic hypoglycaemia (SMBG <2.8 mmol/l) correlated with scores on the abstract /visual reasoning scale ($r=-.39$) (Golden et al 1989). More recently, in a cross-sectional study of 28 type 1 diabetic children (5 diagnosed at <5 years of age who had experienced 1-4 episodes of severe hypoglycaemia; 10 diagnosed >5 years of age who had experienced 1-4 episodes of severe hypoglycaemia; and 13 who had experienced no severe hypoglycaemia) and 28 non-diabetic children matched for age, sex and social background, it was reported that those children with early onset diabetes who had experienced multiple episodes of severe hypoglycaemia had significantly lower scores on tests of psychomotor efficiency and attention. Those children who had been diagnosed after 5 years of age did not differ significantly from the control group. Looking at the tests employed in the neuropsychological test battery in this study it is clear that most are indexing sustained attention which would suggest that the differences between the early-onset diabetes group and the others reflect some form of frontal lobe dysfunction.

Studies in adults

In studies with adult type 1 diabetic subjects there is also evidence that suggests recurrent severe hypoglycaemia may lead to permanent cognitive dysfunction. Bale (1973) tested 100 patients with type 1 diabetes and 100 matched non-diabetic controls on a verbal learning test. Seventeen type 1 diabetic subjects, but no controls, scored in the "brain damaged" range. Further analysis of this data showed that 10 out of 33 patients with a history of at least one admission to hospital with severe

hypoglycaemia had “brain damaged” scores, whereas only one of 23 subjects with no history of severe hypoglycaemia had a score in this range. In reviewing the published data from this study (Bale, 1973), Deary (1993) noted that there was a verbal-performance IQ deficit of about 5 points in those 17 subjects with a history of severe hypoglycaemia. A substantial difference between verbal and performance IQ is traditionally accepted as evidence of organic brain damage because verbal IQ is relatively resistant to such damage whereas performance IQ is more sensitive.

Skenazy and Bigler (1984) found that performance on the Wechsler Adult Intelligence Scale (WAIS) was lower in a group of diabetic subjects compared to either healthy or chronically ill control subjects. The authors also found a 4.5 point decrement in the performance IQ of the diabetic subjects compared with their verbal IQ, but no difference in the two scores in the control groups. Furthermore, in the diabetic group there was a correlation of -0.44 between performance IQ and the number of “insulin-reactions” ($p < 0.04$), but no correlation was observed between verbal IQ and number of “insulin-reactions”.

Wredling et al. (1990) in a well-controlled cross-sectional study compared type 1 diabetic patients with and without a history of recurrent severe hypoglycaemia, on a battery of cognitive function tests. The recurrent severe hypoglycaemia group were slower on two of five Finger Tapping tests, had more reversals on the Necker Cube test, had reduced Forward Digit Span and were slower on the Digit Symbol Substitution test. Langan et al. (1991) interviewed 100 type 1 diabetic subjects diagnosed after 18 years of age to obtain histories of severe hypoglycaemia, and calculated their IQ decrement from the difference between the subject’s WAIS-R performance IQ and estimated pre-morbid IQ from the National Adult Reading Test (NART). A significant correlation (-0.33 ; $p < 0.001$) was found between IQ decrement and reported frequency of severe hypoglycaemia. No significant relationship was found between severe hypoglycaemia and premorbid IQ, memory or verbal fluency scores. Type 1 diabetic patients who had experienced 5 or more

episodes of severe hypoglycaemia had lost 5.8 IQ points more than those with no history of severe hypoglycaemia. Later reports on this cohort have replicated the correlation between frequency of severe hypoglycaemia and IQ decrement (Deary et al. 1992), and have shown that type 1 diabetic patients have lower verbal and performance IQs than matched controls (Deary et al. 1993a). When compared to a group of matched non-diabetic controls, it was evident that performance IQ decrements were associated with recurrent severe hypoglycaemia, whilst a reduction in verbal IQ was related to type 1 diabetes *per se* (Deary et al. 1993a). The major results from the above study were supported by Lincoln et al. (1996) who also investigated the relationship between cognitive impairment and recurrent severe hypoglycaemia in 70 type 1 diabetic subjects aged 24-54 years, all of whom were diagnosed after the age of 18 years. A significant correlation was found between the estimated decline in intelligence score and the frequency of severe hypoglycaemic attacks ($r^2 = -0.30$; $p < 0.01$). Comparison of 11 patients who had experienced 5 or more episodes of severe hypoglycaemia in their lifetime with 35 patients who reported none showed significant differences at the 5% level on scores of memory and information processing (Lincoln et al. 1996). Although the typical degree of cognitive decrement demonstrated in these studies is modest, anecdotal reports have indicated that the professional and social performance of individuals can be affected significantly (Gold et al. 1994a).

Evidence against recurrent severe hypoglycaemia causing permanent cognitive decrements comes from two prospective, large-scale trials of cognitive function in type 1 diabetes. Neither the Diabetes Control and Complications Trial (DCCT) (The Diabetes Control and Complications Trial Research Group, 1997), nor the Stockholm Diabetes Intervention Study (SDIS) (Reichard et al. 1996) have demonstrated any significant, severe hypoglycaemia-related deterioration in cognitive function over time in their respective cohorts. However, the type of patients included in the study must temper interpretation of the DCCT findings.

Patients with a history of severe hypoglycaemia before recruitment were excluded , as were those patients who had experienced a severe hypoglycaemic episode without symptomatic awareness in the two years before the study (i.e. had impaired hypoglycaemia awareness). The follow-up period was short (mean 6.5 years), the patients were highly motivated, of above average intelligence, young and had type 1 diabetes of short duration. Therefore the exposure to severe hypoglycaemia of patients participating in the DCCT may have been significantly less than that experienced by type 1 diabetic patients outside the setting of an interventional prospective clinical trial. In the SDIS Trial (Reichard et al. 1996) it is possible that a Type 2 statistical error may have occurred because the two groups were separated by treatment type rather than by frequency of severe hypoglycaemia, and there was no significant difference between the number of type 1 diabetic subjects who had experienced severe hypoglycaemia in the intensively treated group (86% over 10 years) and the conventionally treated group (73%), although there was a difference in the number of episodes experienced per patient per year (1.06 vs. 0.47 episodes per subject per year). Furthermore, the cognitive test battery used in the SDIS was inadequately sensitive (Deary, 1993).

The relationship between severe hypoglycaemia and cognitive impairment in type 1 diabetic subjects is, therefore, not clearly established. The majority of studies give support to the hypothesis that recurrent severe hypoglycaemia causes a cumulative cognitive decrement, especially in the domain of Performance IQ, and especially in children diagnosed under the age of 5 years. The main concern that has been voiced about the conclusions drawn from these studies is that they mostly reflect cross-sectional data and should therefore be interpreted cautiously with respect to causality. Whilst this may be true it would also be premature to dismiss the findings as they all point in the same direction and indicate that properly designed longitudinal studies in this area are needed.

Neuroimaging and the cerebral effects of severe hypoglycaemia

In the search for more objective evidence of altered brain function in type 1 diabetes following recurrent severe hypoglycaemia Single Photon Emission Topography (SPET) has been used to examine regional cerebral blood flow. Persistent abnormalities in regional cerebral blood flow, measured during euglycaemia, were observed in type 1 diabetic subjects with a history of recurrent severe hypoglycaemia (MacLeod et al. 1994). This was seen to particularly affect the frontal lobes where basal regional cerebral blood flow was elevated (MacLeod et al. 1994). These changes are also observed during acute hypoglycaemia in non-diabetic and type 1 diabetic subjects (MacLeod et al. 1996a). More recently Magnetic Resonance Imaging (MRI) has been used to examine the brain of subjects with type 1 diabetes. A high prevalence of MRI-detected brain abnormalities in type 1 diabetic subjects was noted (Perros et al. 1997). Two principal types of abnormality were demonstrated; brain atrophy and leukoaraiosis. Leukoaraiosis is associated with normal ageing and a variety of medical conditions such as hypertension, demyelination and vascular dementia, and appears to be more prevalent in type 1 diabetic subjects, especially in those subjects with long duration of disease and microvascular complications (Dejgaard et al. 1991). There was a high prevalence of cortical atrophy in the type 1 diabetic subjects who had been exposed to recurrent severe hypoglycaemia (Perros et al. 1997). Five of 11 patients (45%) with a history of severe recurrent hypoglycaemia (mean age 45 years) had evidence of cortical atrophy compared to none of a group of 11 matched type 1 diabetic patients without a history of hypoglycaemia ($p < 0.05$). The MRI findings suggest that cortical atrophy in this relatively young cohort of type 1 diabetic subjects might have developed as a consequence of recurrent severe hypoglycaemia.

Non-cognitive aspects of psychological function during hypoglycaemia

Several early anecdotal accounts have described how hypoglycaemia might effect behaviour and personality (Fischer and Dolger, 1946; Jones, 1947; Wilder, 1943; Murphy and Purtell, 1943). However, it was not until the late 1980s that this area was explored in more detail. Gonder-Frederick et al. (1989) in a study of 34 type 1 diabetic individuals demonstrated an idiosyncratic relationship between mood and blood glucose concentration. Although no significant findings emerged, low blood glucose levels tended to be associated with negative mood states such as 'nervousness'. In a more controlled laboratory setting, Hepburn et al. (1995) measured mood, using the MacKay and Cox Mood Adjective Checklist, every 15 minutes during an insulin infusion which was designed to induce symptomatic hypoglycaemia. Amongst 12 non-diabetic subjects and 15 type 1 diabetic patients there was a significant increase in tense arousal (a measure of anxiety-calmness) and a decline in energetic arousal (a measure of subjective energy), which suggested a state of 'tense-tiredness'.

The use of controlled hypoglycaemia as a biological tool for studying mood-state was first reported by Gold et al. (1995c). This study improved on that by Hepburn et al. (1995) in that it included a euglycaemic control limb and used a hyperinsulinaemic glucose clamp technique to induce more controlled hypoglycaemia. The UWIST Mood Adjective Check List (Matthews et al. 1990) was administered to 24 non-diabetic subjects during hypoglycaemic and euglycaemic steady states using a repeated measures design. In comparison with normoglycaemia, hypoglycaemia was characterised by an increase in tense arousal, a decrease in energetic arousal, and a decrease in hedonic tone (a measure of happiness-sadness). This again suggests the state of 'tense-tiredness'. Thayer (1989) has previously postulated that mood might consist of two principal components, tense arousal and energetic arousal, which would act in opposite directions to

produce a state of tense tiredness. The state of tense-tiredness had been implicated in the development of certain clinical states such as depression. Thayer (1989) indicated that there was a need to develop methods of inducing this mood state to validate the notion of multiple arousal systems underlying subjective reports of mood, suggesting that “tension, energy, and the associated conditions of physiological arousal might seem as variations of the same psychophysiological condition, perhaps differentiated only in terms of subjective labelling” (p.54). The study by Gold et al. (1995c) demonstrated that acute hypoglycaemia could evoke the theoretically important mood state of tense-tiredness, and lent support to Thayer’s (1989) original hypothesis. Furthermore, support was also provided for the validity of the three-dimensional structure of mood proposed by Matthews et al. (1990).

It remains possible that the relationship between blood glucose levels and mood share a common mechanism but that they are not causally related. For instance, individuals who experience a more profound physiological stimulus during hypoglycaemia may be more likely to show a change in mood-state (i.e. those individuals who experience more intense autonomic symptoms [reflecting the catecholamine response to hypoglycaemia] or neuroglycopenic symptoms [reflecting low blood glucose to the brain] during hypoglycaemia may report a negative mood because of the symptoms they are experiencing rather than because of the blood glucose level per se). However, in neither of the aforementioned studies was a relationship between symptom intensity and mood state reported. Also, a recent study (Merbis et al. 1996) of type 1 diabetic subjects with unawareness of hypoglycaemia found that subject’s reported an overall tendency to a more negative mood-state and to increased feelings of anger during hypoglycaemia despite an impaired symptomatic response. This study was small and uncontrolled and so it is not possible to state with certainty that the individual symptomatic response to hypoglycaemia does not influence the mood-state experienced. There is also no direct information on the association between counterregulatory hormone release

during hypoglycaemia and mood-state. It is possible that the change in mood-state results from an effect of these hormones on the brain, although the findings of Merbis et al. (1996), would not be consistent with this. It is of note that a study of adrenalectomized individuals suggested indirectly that adrenaline might be important in the induction of tense arousal (Hepburn et al. 1996).

Recurrent hypoglycaemia and mood-state

There is very little information on the effects of repeated hypoglycaemic episodes on mood-state. Type 1 diabetic patients with recurrent severe hypoglycaemia are reported to score lower on measures of happiness and tend to be more anxious (Wredling et al. 1992). Personality change (an increase in neuroticism and a decrease in extraversion) has been reported in a small group of type 1 diabetic individuals who had long duration of disease and a history of recurrent severe hypoglycaemia (Gold et al. 1994a). Neuroticism has also been found to be higher in type 1 diabetic individuals with self-reported impaired awareness of hypoglycaemia (this is often used as a surrogate measure for frequent hypoglycaemia) (Hepburn et al. 1994). However, all of these studies are small, and cross-sectional, and therefore should be interpreted with caution. Until prospective studies are reported the association between recurrent hypoglycaemia and personality variables and mood remains a speculative one.

PART 1

CHAPTER 3

MENTAL (INFORMATION) PROCESSING

Mental (information) processing refers to the series of successive stages between the appearance (detection) of a physical stimulus and an organism's response to it. One model of this process sees it as a series of separate stages that take up a finite and measurable period of time (Holzman, 1994). This model suggests that each stage can be regarded as a specialised unit that has a specific role in processing of sensory information. The serial nature of this process does not exclude the possibility that simultaneous, parallel processing of information from several input sources may occur. The advantage of this stage model of information processing is that it allows a global process to be divided up into smaller units that may permit analysis.

Recent advances in experimental psychology and psychophysics have lead to the development of psychophysical tests which allow a more specific and detailed examination of mental processing. The following section will briefly review the theoretical background of these tests and the literature that has evolved around them.

Inspection time

Inspection Time (IT) is a psychophysical measure which indexes the efficiency of early visual information processing. The IT task is thought to measure the efficiency with which information in iconic (sensory) memory is transferred to discrimination processes in working memory (Deary et al. 1991). It is a measure of the presentation time required for a subject to achieve a reliable level of accuracy in a simple two-choice visual discrimination task. The stimulus is usually backward masked.

Vickers and colleagues (1972) described the theoretical basis of the IT. They hypothesised that human visual perception functions through a series of quantal 'inspections' of the environment. Therefore, for a stimulus of a fixed magnitude under controlled conditions, the amount of stimulus input time required to make a given discrimination might represent a stable measure of an individual's perceptual performance. The stimulus input time required by a subject to make an accurate two-choice decision on a simple stimulus is called the 'inspection time' of that individual for that particular stimulus (Vickers et al. 1972).

Deficits in early visual information processing have been found in pre-senile Alzheimer's disease (Deary et al. 1991), multiple sclerosis (Kujala et al. 1994), alcoholism (Wilson et al. 1988), head injury (Mattson et al. 1994), following recovery from general anaesthesia (Chittellborough et al. 1992) and in major behavioural disorders such as Schizophrenia and Mania (Green et al. 1994). Various pharmacological agents such as scopolamine (Brandeis et al. 1993), anti-epileptics (Gillham et al. 1988), noradrenergics (Halliday et al. 1994), dopaminergics (Halliday et al. 1987), and anaesthetic agents (Chittellborough et al. 1992) can also affect mental processing speed. Inspection time is known to deteriorate with age (Nettelbeck and Vernon, 1987), and ability on the Inspection Time task correlates with psychometric intelligence (Nettelbeck and Vernon, 1987), and, perhaps more specifically, with fluid-type intelligence tasks (Deary et al. 1991). This suggests that

mental processing speed is an important aspect of general mental ability, and also that it is sensitive to mild cognitive impairment.

Visual Change and Visual Movement Detection

These two psychophysical tasks are based on the work of Philips (1974). In an elegant series of experiments Philips was able to relate a psychophysical model of visual detection of appearances and disappearances directly to changes in the neural activity of cells in the Lateral Geniculate Nucleus (LGN), the first post-retinal visual centre. In the visual detection task an array of light points is displayed, turned off for some time, and turned on again. The task is to detect and locate a point that was in the first display but not the second (disappearance) or vice-versa (appearance). It was shown, by taking intra- and extra-cellular recordings from relay cells of the cat's lateral geniculate nucleus, that performance on this task could be predicted by a model of lateral geniculate neural activity (Philips 1974). It was also observed that some neurones of the LGN discharge when a point of light is turned on in the centre of their receptive field (ON's), and others discharge when a point of light is turned off in the centre of their receptive field (OFF's). Reciprocal cross inhibition occurs whereby on-centre cells can inhibit off-centre cells and vice-versa, and this enhances responses that partly take place already at the retinal level. With stimulus onset, the ON's respond with a transient burst and gradually settle to a maintained level of activity. Because of antagonistic inhibition the OFF's become hyperpolarised at light onset. The resultant inhibition gradually decays, and after offset of the stimulus the OFF's burst into activity that decays gradually, unless the activity is suppressed by the onset of light again. If the onset of light occurs before the latent period of the OFF's has expired, the cell may be hyperpolarised again and never fire at all. Philips (1974) showed that conditions for appearance detection were different from conditions for disappearance detection and that detection was not a function of the comparison of successive 'icons' (i.e. was probably indexing 'pre-iconic')

processing). This work was replicated in a series of experiments over the ensuing years (Royer and Gilmore, 1984 and 1985; Di Lollo and Bourassa, 1983; Stelmach et al. 1987; Wilson et al. 1987; Bourassa et al. 1987; Takakuwa and Callaway, 1990).

The visual change detection and visual movement detection tasks therefore relate to the inspection time task in that both are providing a measure of the early stages of visual information processing. Inspection time provides an index for this process for a stimulus presented in fixed, predictable location whereas the change and movement detection tasks index a wider, unpredictable visual field.

Test of Basic Auditory Capabilities

The Test of Basic Auditory Capabilities (TBAC) (Watson et al 1982) is a battery of auditory discrimination tests which has been used extensively in auditory research, especially that which has explored the relationships between auditory perception and reading disabilities (Watson and Miller 1993). The TBAC was derived from the examination of a large number of subjects with normal hearing (Watson et al 1982). Analysis of 22 different tests suggested 8 subtests that best described the major dimensions of auditory capability, and these 8 subtests were grouped into three primary dimensions of auditory ability: simple auditory discrimination, auditory temporal processing (non-verbal) and speech perception (Watson and Miller 1993, Johnson et al 1987). The TBAC is also thought to provide an overall measure of the speed and efficiency of auditory information processing (Watson and Miller 1993). Performance on the TBAC is unaffected by mid- or high-frequency hearing loss (Christopherson and Humews 1992). The psychometric properties of the TBAC and its test-retest reliability in normal and in elderly hearing-impaired listeners have been validated (Christopherson and Humews 1992).

PART 1

CHAPTER 4

HYPOTHESIS FOR STUDIES

Studies 1 and 2 - Information processing and acute hypoglycaemia

The effect of neuroglycopenia on different aspects of cognitive function has been demonstrated and quantified in studies using a variety of neuropsychological and neurophysiological tests (see chapter 2). In general, these studies have employed measures of general cognitive performance to assess brain functioning during hypoglycaemia. A problem with this approach lies in our limited knowledge of the brain processes involved in most psychometric tests (Anastasi 1990). An example is the Stroop test, which has been the subject of much, detailed research and is used extensively in hypoglycaemia studies and yet for which there is no agreed account of the processes involved in its performance (Deary 1993). The fundamental information processing functions affected by hypoglycaemia therefore remain unknown, because most standard tests cannot be reduced reliably to a specific set of psychological processes. In addition, it is not clear to what extent a significant disruption in general cognitive performance tests provides a useful index of abilities that are important in everyday cognitive functioning (Anastasi 1990). Thus, very little detailed information is available on the effects of controlled hypoglycaemia upon specific information processing abilities. This information is essential to aid our understanding of the relative susceptibility, or importance, of different parts in the sequence of a cognitive process; and to provide information of more direct relevance to insulin-treated patients. Studies 1 and 2 were designed to examine the

effect of acute hypoglycaemia on two fundamental aspects of mental processing, namely; visual and auditory information processing respectively. It was hypothesised that acute hypoglycaemia would lead to a significant disruption in both visual and auditory information processing.

Studies 3 and 4 - Non-cognitive aspects of psychological function during hypoglycaemia

For both studies 3 and 4 it was hypothesised that hypoglycaemia, through its induction of a negative mood state, would lead to an increased expression of negative emotions in the participants. It has long been recognised that some individuals show a marked increase in anger state during hypoglycaemia. However, this has never been systematically studied. Study 3 was designed to examine the effect of acute hypoglycaemia on anger-state using a well validated anger questionnaire, and further to explore possible associations between an individual's anger-trait, general mode of anger-expression, and change in anger-state. Similarly study 4 was designed to extend previous work on acute hypoglycaemia on mood state. It was hypothesised that acute hypoglycaemia through the induction of a state of *tense-tiredness* would lead to a negative emotional state, which would cause individuals to make more negative appraisals of a life event.

Study 5 – A comparison of the symptoms of hypoglycaemia induced using a continuous insulin infusion or a one-step hyperinsulinaemic glucose clamp in non-diabetic and IDDM subjects.

Previous studies have suggested that different techniques for inducing hypoglycaemia may not evoke the same symptomatic response. It has also been suggested that the Edinburgh Hypoglycaemia Scale, which has been derived from type 1 diabetic subjects in the community, may not be applicable to experimental hypoglycaemia. This study sought to answer these questions by analysing the symptom reports of a large number of type 1 diabetic and non-diabetic subjects who

have participated in experimental hypoglycaemia research at the Royal Infirmary of Edinburgh.

Study 6 – The symptoms of hypoglycaemia experienced by children with type 1 diabetes.

The application of statistical techniques such as Principal Components (Factor) Analysis to examine symptoms of hypoglycaemia in large groups of type 1 diabetic adults lead to the development of the Edinburgh Hypoglycaemia Scale (Deary et al. 1993b). This measure of hypoglycaemia symptoms provides a possible means by which standardisation may be achieved across research centres. However, it is not known whether the symptoms of hypoglycaemia in children with type 1 diabetes can be classified in the same way. The literature would suggest that when they are experiencing hypoglycaemia behavioural change is more apparent in type 1 diabetic children. This study was designed to apply similar statistical techniques to the analysis of hypoglycaemia symptom reports from type 1 diabetic children in an attempt to determine more accurately the nature of the symptoms of hypoglycaemia experienced by this group of patients.

PART II

METHODS

Methods

Ethical Permission

The Lothian Health Board Ethics of Medical Research Subcommittee for Medicine and Clinical Oncology granted permission for each of the studies. Written consent was obtained from all subjects following a detailed explanation of the nature of the studies.

Subjects

The details of criteria for selection of subjects for each study are explained in the individual study chapters.

Laboratory studies of hypoglycaemia

The laboratory studies involving experimentally-induced hypoglycemia were conducted in the Department of Psychology, University of Edinburgh, during the period 1994-5.

The hyperinsulinaemic glucose clamp technique: In studies 1-4 hypoglycaemia was induced and maintained using a modified manual hyperinsulinaemic glucose clamp (DeFronzo et al. 1979). This method uses a fixed rate insulin infusion and a variable rate 20% dextrose infusion to manipulate the blood glucose. Insulin is initially infused at a rate of 150 mU/m²/min before being gradually ramped down, over a period of 10 minutes, to a rate of 60 mU/m²/min, at which it is then maintained. This loading dose saturates the insulin receptors to facilitate subsequent manipulation of blood glucose. An infusion of 20% dextrose is commenced about 5 minutes after the insulin infusion is started, initially at a rate of about 1 ml/kg/hour. The subsequent rate of dextrose infusion is determined by the arterialised blood glucose

concentrations measured by the bedside every 5 minutes. In all experiments a period of stable euglycaemia is first established to enable baseline measurements to be taken. To then induce hypoglycaemia the dextrose infusion is stopped for 5 minutes then recommenced at half the rate that was required during stable euglycaemia. The dextrose infusion is then adjusted as required to maintain the predetermined level of hypoglycaemia. In the following experiments the blood glucose is initially stabilised at euglycaemia (5.0 mmol/l) and then allowed to fall rapidly to a level of 2.6 mmol/l (the predetermined level of hypoglycaemia). This is the one-step euglycaemic-hypoglycaemic clamp and allows the investigator to provide a reproducible fixed level of hypoglycaemia for a predetermined time and an identical stimulus with which to compare subjects.

The hyperinsulinaemic glucose clamp produces plasma insulin levels that are supraphysiological and this may have an influence on the outcome of experimental studies employing this technique. The role of insulin in the counterregulatory and cognitive responses to hypoglycaemia has been discussed in an earlier section of this thesis. However, by including a hyperinsulinaemic euglycaemic control in each study design it is hoped that this potential variable is controlled for. It is also a criticism of this technique that, in the light of recent evidence of the variability of individual responses to hypoglycaemia (Gonder-Frederick et al. 1994), it is not in fact providing an identical stimulus to each individual (i.e. a fixed hypoglycaemic stimulus of 2.6 mmol/l may reflect different degrees of hypoglycaemia between individuals in whom the thresholds for cognitive dysfunction vary). However, as discussed in a previous section, the threshold for cognitive dysfunction is probably less variable than that for the counterregulatory response to hypoglycaemia, and by choosing a degree of hypoglycaemia that is sufficiently below the 'accepted' threshold for cognitive dysfunction it is hoped that most individuals will show a degree of cognitive change. Finally, it must be recognised that the technique produces a stable period of hypoglycaemia that is not analogous to the 'real-life'

experience of hypoglycaemia in type 1 diabetic individuals, and therefore the interpretation of data from clamp experiments must be made with caution. Despite these reservations, the hyperinsulinaemic glucose clamp is the only method available for reliably maintaining the blood glucose at predetermined concentrations for prolonged periods of time, sufficient to allow the administration of cognitive test batteries.

Experimental procedure for clamp studies: Following a light breakfast at 0700 h subjects were asked to attend the department at midday, and a Teflon cannula was inserted into an antecubital vein in the non-dominant arm under local anaesthesia (lignocaine 1%). This was used to infuse a fixed rate of human soluble insulin (Humulin S, Eli Lilly, Indianapolis, USA) and a variable infusion of 20% dextrose as described above. Insulin was infused at a constant rate of $60 \text{ mU/m}^2/\text{min}$ using an IMED Gemini PC1 pump. Dextrose was infused using an IVAC Site Saver pump. The rate of dextrose infusion was adjusted according to the arterialised blood glucose concentration measured at the bedside (Yellow Springs Instrument 2300 Stat, Yellow Springs, Ohio, USA). A second cannula was inserted in a retrograde direction into a vein on the back of the hand. The cannulae were flushed regularly with heparinised 0.9% saline. The hand was placed in a heated Plexiglas box (60°C) to arterialise venous blood. Arterialised venous blood samples were initially obtained at three-minute intervals, then at five-minute intervals once a stable blood glucose concentration had been achieved. Warming the hand allows shunting of the blood from the arterial to the venous system. This “arterialised” venous blood glucose has been shown to approximate very closely with true arterial blood glucose concentrations (Liu et al. 1992). This is necessary because venous blood glucose concentrations are highly variable, particularly in combination with the glucose clamp technique (Liu et al. 1992).

The subjects were not informed of their blood glucose concentrations during the experiments. The blood glucose was initially stabilised and maintained at 5.0 mmol/l in all the clamp studies and then either allowed to fall to a hypoglycaemia plateau (2.6 mmol/l) or continued on at euglycaemia (5.0 mmol/l).

Choice of degree of hypoglycaemia: A level of hypoglycaemia was chosen that would ensure the threshold for cognitive dysfunction had been reached in most individuals, and which would be tolerated safely by the subjects, for the duration of at least one hour. Based on a review of the literature (see Part I, pp.36-67), and previous experiments conducted in our laboratory, it was decided to aim for a degree of hypoglycaemia of approximately 2.5 -2.6 mmol/l.

Mental (information) processing tests

(A) Visual information processing tests

Inspection Time.

The Inspection Time (IT) Test used in this study was a simple two-choice discrimination task. IT is a measure of the speed of the early stages of visual information processing (Vickers et al. 1972).

The object of the test is to determine the stimulus duration required by a subject in order to reach a given level of correct responding in a very simple discrimination task. In the IT task used here the subject is required to indicate which of two parallel vertical lines, of markedly different lengths, is the longer. During the test the experimenter varies the stimulus duration, with briefer duration's being more difficult. Only the correctness of responses at different stimulus durations is examined. Response speed is *not* recorded and the subject is instructed and encouraged to respond at leisure to achieve maximum accuracy. The IT stimuli and backward masks have been described in detail by Deary et al. (1991). A mask is designed to prevent the further processing of information from a briefly presented stimulus. The stimulus presentation unit was a box that carried a 16x16 array of circular red light-emitting diodes (LEDs). This display area (61 mm square) was built up from four Siemens PD 1165 display modules, each of which had an 8x8 grid of LEDs of 0.11 inch diameter on 0.15 inch centres. All stimuli were composed of these LEDs. The cue, which preceded the IT stimulus, was an inverted U-shape, 16mm across by 14mm high, which shared the same crossbar as the IT stimulus. In the IT stimulus the long line (29 mm) and short line (14 mm) were joined at the top by a crossbar (16 mm). Thus the vertical lines were aligned at the top. The backward mask was formed by lines 40 mm long and 10 mm wide (i.e. the masking lines were wider and longer than the stimulus lines and completely covered them).

The stimulus presentation unit was controlled by a BBC computer, which also collated the response data.

Each subject carried out all the IT test sessions under the same lighting conditions and in the same room, to ensure that stimulus contrast remained stable. A 300 msec warning cue was presented at the start of each trial. One second after onset of the cue, the IT stimulus was presented for the appropriate duration, which ranged from 1 to 400 msec. After IT stimulus offset the backward mask was presented immediately to prevent further processing of the stimulus. The duration of the mask was adjusted as stimulus duration varied so that stimulus plus mask duration remained at 600msec. After the mask was turned off the subject was required to respond by indicating which stimulus line was the longer. Responses were made on two buttons linked to the stimulus presentation unit. There was no feedback as to the correctness of the response. In this study a response from the subject initiated the next trial (i.e. no reaction time was taken and the subject was continually reminded that the emphasis was on accuracy rather than speed of response).

The IT test algorithm used measured the stimulus presentation time (in msec) required by each subject to achieve 85% accuracy in responding (50% representing chance responding). Stimulus presentation times were determined according to the PEST adaptive staircase algorithm (Taylor and Creelman, 1967). Starting at a presentation time of 200 msec with a minimum of five trials per step, the first step size was 75 msec. The step size was halved with each reversal and the stopping step size was 1 msec. Most subjects required about 90-130 trials to reach their IT. This algorithm has the advantage of using reliability of response as its stopping criterion, and provides a reliable index of visual information intake efficiency (Deary et al. 1991).

Visual Change Detection (VCD).

This test is based on the work of Phillips (1974) on change detection, as described by Wilson et al. (1988). This test, like IT, assesses the speed of early visual processing. However, in this task the subject must identify the locus of a discrete change in a large array of homogeneous stimuli. Therefore, whereas IT measures perceptual speed for detecting a difference in a stimulus occurring at a specific, predictable location, the VCD task requires the subject to attend to a wider stimulus field, so emphasising parallel processing. However, like IT, it is the *timing* (i.e. speed) of the stimulus change that is manipulated, and in this task, as with IT, the subject is allowed to respond without any pressure of time.

The stimulus display used for this test consisted of an array of 49 small rectangles on a computer monitor screen to which, after a variable interval, a single (target) rectangle was added. The subject's task was to identify this additional rectangle. The experimenter can manipulate the time interval between the onset of the 49-rectangle array and the target; shorter intervals are more difficult. The display was generated by randomly lighting 49 of 100 *potential* rectangles in a regular 10x10 array upon a computer screen. The overall array size was 185 mm by 105 mm; each rectangle measured 5mm vertically by 3mm horizontally and were non-contiguous. The cue, which preceded the stimulus by a fixed interval, was a circle located in the centre of the screen. The time intervals employed between the onset of the array and the onset of the target were either: 14, 28, 42, 56, 70 or 86 msec. A response was made by touching the screen on the relevant rectangle, and the subject was given feedback on each trial. In this test the subject initiated the next presentation, and, as with IT, was reminded that *accuracy of responding* was being measured and *not* reaction time. Each subject was tested on a random block of 60 presentations; 10 trials of each of the 6 different stimulus durations were presented at random. A total score of accuracy of responding was obtained.

Visual Movement Detection (VMD).

In this task a subject's efficiency of detecting the movement of a single stimulus in a large array is assessed (Philips 1974, Wilson et al. 1988). The VMD resembles the VCD task in all respects except one: the target rectangle, rather than appearing after the rest of the array, appears with the array but, after a variable interval, moves to the right or left by a distance identical to its width (i.e. 3 mm). As with the test of visual change detection this test display was generated by randomly lighting 50 rectangles in a potential 10x10 array. The array measured 185 mm by 105 mm, and each rectangle measured 5mm vertically by 3 mm horizontally. After a variable interval one (target) rectangle was deleted and re-displayed 3 mm away horizontally, thus creating the subjective sensation of sudden movement. The subject's task was to point to the rectangle that appeared to move. The cue was the same as that used for the visual change detection task. The interval between the onset of the array and the movement of the target rectangle ranged from 14-86 msec as for the VCD task. The subject responded by touching the computer screen over the rectangle thought to be the target. A random block of 60 presentations (10 trials of 6 different stimulus durations) was also employed in this test, and the total number of correct responses was obtained and used as the score.

(B) Auditory information processing tests

The tests used to examine different aspects of auditory information processing were from the Boys Town Institute Test of Basic Auditory Capabilities [TBAC] (Watson et al. 1982).

Each of the discrimination tests used in this battery is conducted using the same psychophysical method, with six levels of the independent variable. The tests are recorded on audiotape together with detailed instructions for the subjects. The psychophysical method is a modified two-alternative forced choice procedure. A

standard comparator stimulus precedes the two stimuli of the two-alternative, forced choice presentation, yielding three stimuli in total for each item. The subject's task is to identify which of the two stimuli following the standard was different from it. This psychophysical procedure thus involves only two types of trials, SDS and SSD (S = standard stimulus, and D = different stimulus). The subject's task is to identify D. This method minimises demands on attention and memory, while providing a valid test of auditory processing abilities (Watson et al. 1982). Trials are always presented in a series of six steps of the independent variable, in ascending difficulty. In the test battery employed in the present study a total of 72 trials was used for each sub-test. The stimulus tones are all gated with 5-msec rise-decay times to minimise onset and offset transients.

The studies were performed in a sound controlled listening room with an ambient sound level of 30-32 dB. The TBAC was recorded on audiotape and played to the subjects on a Levers-Rich Mastering Machine with a Sony Mixer. Subjects heard the tests via Bayer Dynamic DT 100 headphones. The tasks were performed at a sound level of 67 dB (mean of 10 recordings using a regularly calibrated Castle Associates Sound Level Meter).

The TBAC contains eight sub-tests, of which four sub-tests were used. These examined two categories of auditory processing; simple auditory processing (pitch discrimination, single-tone loudness, single-tone duration), and auditory temporal processing (non-verbal; temporal order discrimination) (Watson and Miller, 1993; Watson et al. 1982).

Pitch discrimination: The standard is a 1-kHz, 75-dB, 250-msec tone. The values of the frequency differences range from 2 to 256 Hz.

Single-tone loudness: The standard is again a 1-kHz, 250-msec tone at 75-dB.

Increments in level, expressed in decibels, range from 0.5 to 8.0 dB.

Single-tone duration: The standard is a 1-kHz, 75 dB, 100-msec tone. Increments in duration range from 8 to 256 msec.

Temporal order for tones: The task is to discriminate the order in which two tones are presented, one of which is 550Hz, and the other 710 Hz. The duration of the two tones is varied from 20 to 200 msec. The tones are presented without a gap between them and are preceded and followed, without gaps, by a 'leader' and 'trailer', consisting of 100-msec, 625 Hz tones.

Psychometric tests of general cognitive function

Two tests of general cognitive performance, known to be affected by moderate hypoglycaemia (Deary, 1993), were included in the test battery. Both the Digit Symbol and the Trail Making B test have a closer relationship to 'fluid' rather than to 'crystallised' (vocabulary-oriented) cognitive ability. These tests were included to provide an indication of general brain functioning during controlled hypoglycaemia.

Digit Symbol (DSS). This is a performance sub-test of the Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981). Nine digits are represented by nine different symbols, and subjects are required to write down the appropriate symbol for each in a given array of numbers over a fixed time period. The score is the total number of correct symbols drawn over this time period.

Trail Making Test B (TM-B). This is a divided attention task from the Halsted-Reitan Neuropsychological Battery (Reitan et al. 1974). The subject has to connect correctly an alternating series of numbers (1-13) and letters (A-L) in their respective orders as quickly as possible. The score is the time taken to complete the task.

Tests of mental ability

Prior to enrolment for each of the experimental studies, the general level of mental ability was assessed in each individual. The Alice Heim 4 test (AH4; Heim, 1970) and the National Adult Reading Test (NART, Nelson and Willison, 1992) were used. The AH4 test is divided into two parts, the first part of the test provides an assessment of verbal and numerical skills and the second an assessment of visuospatial skills. The subject is asked to complete as many questions as possible in ten minutes for each part. A choice of answers is provided for each question. The test does not provide a measure of IQ, but does provide a good measure of global cognitive ability. The maximum score is 65. The NART assesses premorbid IQ, that is the peak level of mental ability attained by a subject prior to any cognitive deterioration. In this task the subject is asked to pronounce 50 irregular English words (e.g. ache, depot) and the number pronounced correctly is the score obtained. The NART has been validated against the Wechsler Adult Intelligence Scale and has been shown to correlate closely with this test in subjects without reason for cognitive decline (Crawford, 1992)

Assessment of symptoms of hypoglycaemia

A questionnaire was used to establish the symptoms of hypoglycemia that the subjects were experiencing. The questionnaire was given to the subjects at the appropriate time-points described in each study. This questionnaire has been developed from previous experimental and clinical studies (Hepburn et al. 1991a, Hepburn et al. 1992, Deary et al. 1993b). Hypoglycaemia symptoms were classified as *autonomic* (palpitations, sweating, shaking and hunger), *neuroglycopenic* (confusion, drowsiness, odd behaviour, speech difficulty and inco-ordination) or *non-specific* (nausea and headache) (Edinburgh Hypoglycaemia Scale, Deary et al.

1993b). Each symptom was graded on a scale of 1 to 7 (1 = not present, 7 = very intense). The total for each sub-group of hypoglycaemic symptoms was calculated.

Questionnaires used in studies on non-cognitive effects of hypoglycaemia

Measures of anger state, trait and expression.

The State-Trait Anger Expression Inventory (STAXI) (Spielberger, 1991) is designed to provide easily administered and objectively scored measures of anger experience and expression. The STAXI has been used to examine the relationship between anger expression and systolic blood pressure (Johnson et al. 1987a; Johnson et al. 1987b), and to investigate the psychological functioning of patients with post-traumatic headache (Ham et al. 1994). The STAXI comprises three sub-scales; anger-state, anger-trait and anger-expression, the scoring and interpretation of which have been explained in detail (Spielberger, 1991). Anger-state is a measure of the degree of anger experienced by an individual at the time of questioning. A list of 10 questions is provided which includes questions such as: 'I am furious', 'I feel irritated', and 'I feel angry'. Subjects are not under pressure of time to respond. Scores for each question are graded from 1-4 (1= not felt at all, 4= very intense feeling). The total score for each subject is then calculated. This questionnaire was given to the subjects during the study conditions (euglycaemia and hypoglycaemia) only, and not shown to them prior to this.

The anger-trait measure is based on a series of questions which ask the subject to describe how they generally feel. Persons high in trait-anger frequently experience angry feelings and often feel they are treated unfairly themselves. Measures of anger expression are derived from a 24-stem questionnaire, which asks subjects to indicate how they generally react or behave when they are angry or furious. Separate scores are obtained for the degree to which subjects generally suppress angry feelings (AX/In); express angry feelings as aggressive behaviour (AX/Out); and the degree of

control they tend to exert over angry feelings (AX/Con). From these three individual scores a measure of anger-expression is obtained (AX/EX).

UWIST Mood Adjective Check-list (UMACL (Matthews et al. 1990)).

The adjectives used to characterise the mood dimensions used in this questionnaire were graded by intensity on a scale of 1 to 4 and were classified into three groups: hedonic tone, tense arousal and energetic arousal. Higher scores indicated a high *happiness* rating for hedonic tone, a high *anxiety* rating for tense arousal, and a high *energy* rating for energetic arousal. The maximum score that could be obtained for each of the three mood components was 32, and the minimum score was 8.

Appraisal of Life Events Scale (ALE (Ferguson et al. 1997; Ferguson and Cox, 1993; Ferguson et al. 1998)).

This questionnaire consists of 16 adjectives relating to the three appraisal dimensions described previously: threat, challenge and loss. Questionnaire items were graded by intensity on a scale of 1 to 4 (1=not very intense experience and 4=very intense feeling). A high *challenge* score means the subject is appraising a situation as allowing for personal growth and development. A high *threat* score indicates that a life problem is being appraised as potentially threatening, physically harmful, and generating anxiety. A high *loss* score suggests the potential for personal suffering. The ALE was completed by subjects during study conditions (euglycaemic, hypoglycaemia) only, and not during either baseline.

Eysenck Personality Questionnaire-Revised, Short Form (EPQ-R (Eysenck et al. 1985)).

The EPQ-R short form consists of 48 questions, 12 relating to each of the three major personality dimensions of neuroticism, psychoticism and extraversion, and a further 12 questions making up a lie scale to assess the tendency to give desirable answers. Participants answer yes or no to each question. The scores are then summed

for each dimension. The EPQ-R was completed by subjects during study conditions (euglycaemia, hypoglycaemia) only, and not at each baseline.

Visual acuity and contrast sensitivity tests

Vision acuity was examined by a series of standard clinical tests. An assessment of static contrast sensitivity was also taken to provide evidence for any subtle changes in peripheral vision.

Visual Acuity for each eye was assessed using a standard Snellen Chart positioned 6 m from the subject.

Visual near-point (binocular) and *reading acuity* (monocular) were assessed using a Royal Air Force Meter (Clement Clarke International Ltd, Essex, England).

Stereoscopic Vision, a measure of the ability of subjects to judge the relative distances of objects by means of binocular vision, was assessed using Stereo-test Circles (Stereo-Optical Company Limited, Chicago, Illinois, USA).

Contrast Sensitivity (static) was measured using the Cambridge Low Contrast Gratings (Clement Clarke International Ltd, Essex, England). In each item of this test the subject views two adjacent pages of a booklet positioned at a distance of 6 m, only one page of which contains horizontal lines (a grating). Each 'line' in the grating is composed of small black dots on a white background separated from each other by equal distances. Viewed from a distance the subjective impression is of grey lines with white spaces between them. The opposite page of the booklet has the same number of dots evenly dispersed (i.e. not as lines). Here the subjective impression, when viewed from six metres, is of a blank page. The subject's task is to identify the page that contains the lines (the grating). The task is very difficult when

the lines are composed of few, widely spaced black dots, where the impression is of very faint grey lines on the page. Therefore, by varying the number of dots, and the distance between them, a series of gratings are produced with different levels of contrast. The gratings have 11 levels of difficulty, all with the same spatial frequency of 4 cycles/degree. In this study the subject was presented with a block of 50 presentations (10 trials of each of the five most difficult gratings (contrast % 0.37, 0.27, 0.19, 0.14, and <0.14)) in random order, and the total number correct was used as the score. The lighting in the room was adjusted so that the luminance of the non-grating plate in the demonstration pair (contrast = 13 %) was 100 cd/m². This was kept constant for all visual assessments.

Statistical methods

The individual tests employed are detailed in the relevant study chapters. All statistical procedures were performed using an IBM computer with software package SPSS-PC+.

Power analysis

The calculation of the sample size required to achieve a power of 80% in the studies presented in this thesis is based on the following formula:

$$n > 2[(z_{2\alpha} + z_{2\beta})\sigma/\delta]^2$$

Specifying an α of 0.05 and a power of 80% and assuming that the critical interval (δ) is the same as the standard deviation (σ) then it can be calculated that at least 16 subjects would be required in each study ($z_{2\alpha}$ and $z_{2\beta}$ are obtained from standard tables).

PART III

STUDIES INVOLVING EXPERIMENTAL INSULIN- INDUCED HYPOGLYCAEMIA

PART III

STUDY 1

Visual information processing during acute hypoglycaemia

That moderate hypoglycaemia can cause general cognitive dysfunction has now been established using neuropsychological and neurophysiological tests. However, as I have already indicated, our knowledge of the brain processes involved in most psychometric tasks is limited (Anastasi, 1990). This is important because one cannot infer from studies employing these tasks the actual functional impact of hypoglycaemia on more fundamental brain processes. The speed and efficiency with which the brain is able to process information received from the visual sensory system is essential to normal brain functioning, and may provide an index of mental ability in general (see pp 78-79). Advances in psychophysics have led to the development of tests which provide a much more accurate index of visual information processing. This study was designed to examine the impact of moderate hypoglycaemia on visual information processing in non-diabetic individuals.

Acute controlled hypoglycaemia does have an effect upon the eye with documented changes in intra-ocular pressure (Frier et al. 1987) and anterior chamber dimensions (Hepburn et al. 1993), but it is not known whether these physical changes translate into clinically relevant abnormalities of vision. Blurring of vision and diplopia are recognised features of acute hypoglycaemia (Hepburn, 1993). When symptom profiles reported during acute insulin-induced hypoglycaemia are analysed, blurring of vision clusters together with the neuroglycopenic symptoms of hypoglycaemia (Hepburn, 1993). Corrected visual acuity does not appear to deteriorate during

insulin-induced hypoglycaemia (Harrad et al. 1985), nor is there evidence of a significant change in colour vision (Hardy et al. 1995). These findings are inconclusive and afford data of limited practical relevance because real-world visual perception involves making decisions about transitory and low contrast stimuli. An important example of such a task is driving, and deleterious effects of hypoglycaemia on driving ability have been found using a driving simulator (Cox et al. 1993).

Contrast sensitivity provides a more subtle measure of unspeeded visual function (Di Leo et al. 1992) than standard acuity tasks, and is more relevant to everyday human visual perception. Contrast sensitivity provides a measure of the amount of contrast required to detect a visual target. Sub-clinical visual dysfunction is more likely to be detected by degrading the stimulus (visual target) intensity using this more demanding visual assessment (Di Leo et al. 1992).

The aim of the present study was therefore to examine two important aspects of visual perception during controlled hypoglycaemia, viz. speed of iconic processing and contrast sensitivity. Standard tests of general cognitive performance gave a measure of the degree of general cognitive disruption during hypoglycaemia. Simultaneous standard measurements of visual acuity were recorded.

Methods

Subjects

Twenty (18 male, 2 female) healthy non-diabetic human subjects were studied. All subjects had a visual acuity (measured by a Snellen chart) of 6/6 or better. The subjects were aged 26 (23-30) years (median (range)) and had a Body Mass Index of 23 (19-26) kg/m². All of the subjects had above average intellectual ability as assessed by the National Adult Reading Test and the Alice Heim 4 Test (standard

tests of general intellectual ability). The subjects did not have a previous medical history or a family history of diabetes, nor were they taking regular medication. Subjects were recruited by advertisement, and were not paid for their participation in this study. Each subject was informed that they would be required to attend the department on three separate occasions. On two of these occasions they would undergo the experimental procedure during which they would either be kept at euglycaemia or rendered hypoglycaemic. Subjects were made aware that they would undergo both study conditions but would not be informed as to which condition was being performed on each study day. The local medical ethics advisory committee approved the study, and written consent was obtained from all subjects.

Experimental Procedure

The subjects were studied on three separate occasions, each at least two weeks apart. The initial visit was to familiarise the subjects with the tests that would be used during the experimental condition. During familiarisation subjects completed all of the psychometric tests, the order and duration of which were the same as that of the experimental condition. Familiarisation was used to help minimise any practice effects that might occur. The results from this session for each individual subject were discarded. In the two subsequent visits to the laboratory subjects underwent a hyperinsulinaemic glucose clamp procedure (described on pages 86-88).

The study design is shown in Figure 3.1.1. Each subject underwent two laboratory sessions. On each occasion the arterialised whole blood glucose concentration was initially stabilised at 5.0 mmol/l (baseline phase) for one hour. In one study session hypoglycaemia (2.6 mmol/l) was then induced and maintained for one hour, and in the other the blood glucose concentration was maintained throughout at 5.0 mmol/l (euglycaemia). An interval of 20 minutes was interposed between baseline and the hypoglycaemic or euglycaemic phases to allow achievement of the new glycaemic level. Subjects were not informed of their blood glucose level at any time during the

laboratory sessions. During each phase of the study (each baseline, euglycaemia and hypoglycaemia) the subjects were asked to complete the test battery (see below). During each study phase the blood glucose was stabilised for ten minutes at the target glycaemic level before the assessment of information processing. The subjects underwent the experimental conditions in a counterbalanced fashion (i.e. half the subjects underwent hypoglycaemia first followed by the euglycaemic control condition, and half the reverse).

Test battery

During each phase of the study (each baseline, euglycaemia and hypoglycaemia) the subjects underwent tests of:

Visual Information Processing

- 1 - Inspection Time [IT] (Vickers et al. 1972).
- 2 - Visual Movement Detection [VMD] (Phillips, 1974).
- 3 - Visual Change Detection [VCD] (Phillips, 1974).

General Cognitive Function

- 4 - Digit Symbol Substitution test [DSS] (Wechsler, 1981).
- 5 - Trail Making Test B [TM-B] (Reitan and Davison, 1974).

Visual Acuity and Contrast Sensitivity

- 6 - Visual Acuity
- 7 - Visual Near Point
- 8 - Stereoscopic vision
- 9 - Contrast Sensitivity

Hypoglycaemia symptoms.

- 10 - Edinburgh Hypoglycaemia Scale (Deary et al. 1993b).

The tests used in the battery are described in detail in Part II. Subjects completed the tests in the same order during each study condition, and the battery took between 40-45 minutes to complete.

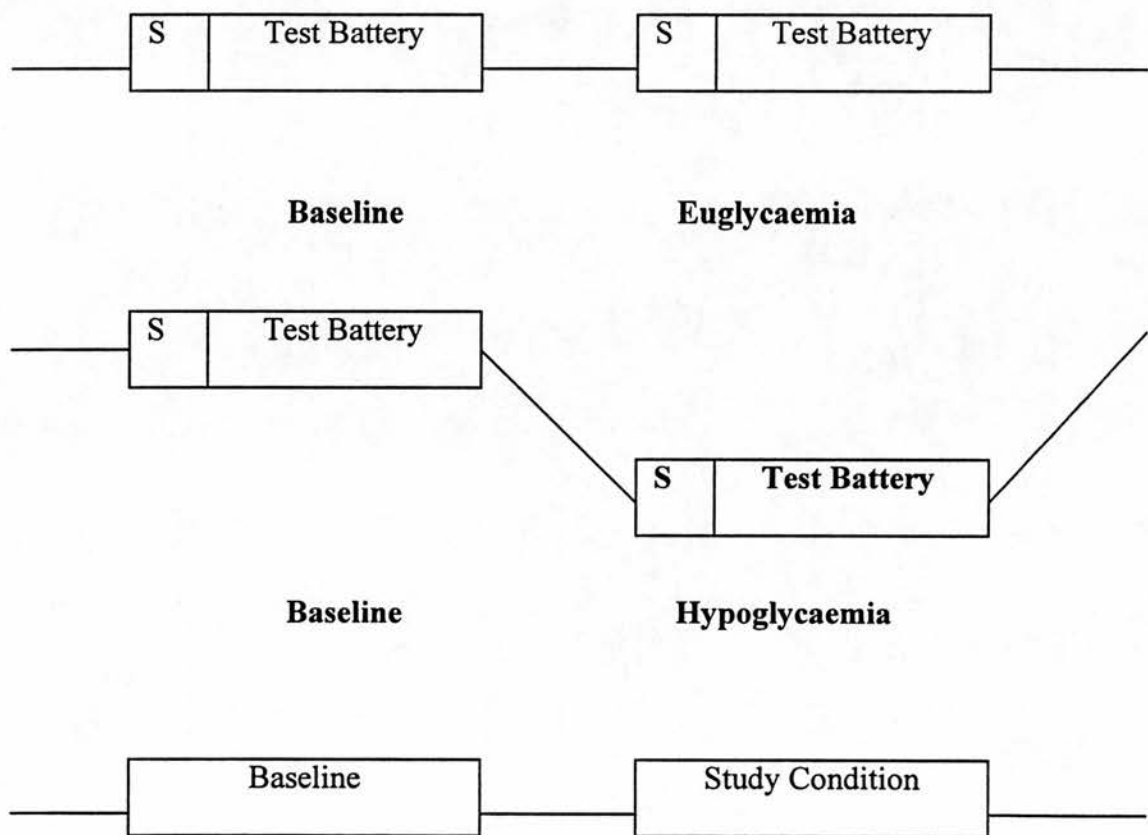
Statistical analysis

The results were analysed independently for each measure of visual function, tests of general cognitive function, and information processing tests. A mixed model Analysis of Variance (ANOVA) was used with order of session as a between subjects factor with two levels (euglycaemia-hypoglycaemia or hypoglycaemia-euglycaemia), and study type as a within subjects factor with two levels (euglycaemia vs. hypoglycaemia). Test results at baseline were used as variable covariates in each analysis.

Due to a computer fault on a single occasion one of the subjects was unable to complete the assessment of visual change detection and visual movement detection during hypoglycaemia.

Figure 3.1.1.

This diagram represents the study design. Each subject underwent both euglycaemic and hypoglycaemic study conditions on separate days. Once stable glycaemic levels had been achieved a period of stabilisation (S) followed prior to subjects completing the test battery. The test battery was completed during baseline(s) *and* study conditions.



Results

Blood glucose

Overall mean \pm S.D plasma glucose during the hypoglycaemic clamp was 2.60 ± 0.1 , and 4.98 ± 0.05 during the euglycaemic clamp (Fig.3.1.2).. The coefficient of variation in blood glucose was 0.5% and 0.6% for the euglycaemic and hypoglycaemic clamps respectively.

The initial statistical analysis revealed that no significant order effects (asymmetrical transfer effects) had occurred for any of the outcome variables in this study.

Symptoms

There was a significant increase in total autonomic symptom scores ($F = 33.31$, df 1,18, $p < .001$) and total neuroglycopenic symptom scores ($F = 12.86$, df 1,18, $p < .005$) during the hypoglycaemia condition of the study (Table 3.1.1).

Hypoglycaemia-associated non-specific malaise symptom scores showed a tendency to increase though this did not achieve a conventional level of significance ($F = 4.25$, df 1,18, $p = .055$). Sub-group analysis is based upon symptoms from the Edinburgh Hypoglycaemia Scale (Deary et al. 1993b).

General cognitive function

In the present study scores achieved on the DSS were significantly lower ($F = 69.97$, df 1,18, $p < .001$), and times taken to complete the TM-B were significantly longer ($F = 4.66$, df 1,18, $p < .05$) during hypoglycaemia when compared with euglycaemia (Table 3.1.1). This confirms that these standard measures of general cognitive dysfunction were significantly affected at a blood glucose of 2.6 mmol/l.

Visual acuity and contrast sensitivity

No significant changes were observed in visual acuity or visual near point during

hypoglycaemia (Table.3.1.1). Visual acuity, for both eyes, tested separately, was not significantly affected by hypoglycaemia ($F = 2.22$, $df\ 1,18$, $p = ns$, and $F = 1.08$, $df\ 1,18$, $p = ns$, respectively). Visual near point for binocular vision ($F = 0.81$, $df\ 1,18$, $p = ns$), and reading point, for both eyes tested separately ($F = 0.00$, $df\ 1,18$, $p = ns$, and $F = 0.16$, $df\ 1,18$, $p = ns$ respectively), showed no significant changes during hypoglycaemia. Stereoscopic vision was also unaffected ($F = 0.00$, $df\ 1,18$, $p = ns$). Therefore, standard tests of visual acuity were unaffected during controlled hypoglycaemia, indicating that no disruption in vision, as detectable by these tests, had occurred despite a significant and concurrent deterioration in cognitive performance.

Although visual acuity for highly contrasting symbols was unaffected during moderate hypoglycaemia, a significant deterioration was observed in the ability of the subjects to detect differences in contrast ($F=13.70$, $df\ 1,18$, $p<.005$) (Fig.3.1.3). This implies that visual dysfunction is demonstrable when the visual system is stressed by symbols of low visual contrast (i.e. by degradation of the stimulus).

Visual information processing

Acute insulin-induced hypoglycaemia caused a significant and marked deterioration in visual information processing ability (Table 3.1.1). Visual inspection time (IT) for the study group increased (i.e. the presentation times required to discriminate between the two arms of the stimulus were longer) during moderate hypoglycaemia ($F=8.35$, $df\ 1,18$, $p<.05$) (Fig. 3.1.4). Hypoglycaemia resulted in an impaired ability to detect visual change ($F=13.34$, $df\ 1,17$, $p<.005$) (Fig.3.1.5), and an impaired ability to detect visual movement ($F=14.19$, $df\ 1,17$, $p<0.005$) (Fig.3.1.6). These results indicate that moderate hypoglycaemia significantly disturbs the early stages of visual information processing both for focused stimuli and for stimuli presented in a wider visual field.

The group's mean (\pm SEM) total score achieved during euglycaemia and hypoglycaemia for each stimulus duration on the VCD and VMD tasks (Fig. 3.1.7 and Fig. 3.1.8), and for each level of difficulty on the contrast sensitivity task (Fig.3.1.9) demonstrate a shift in the response curve along the x-axis during hypoglycaemia; the steepness of the curve was not affected. This finding indicates that hypoglycaemia raises detection thresholds in each of the visual information processing tasks (Wilkins et al. 1988). A change in subjects' criterion (their willingness to report a weak sensation) or neural 'noise' would be expected to alter the steepness of the curve.

Table 3.1.1. Visual information processing, visual acuity, and general cognitive performance during baseline and study conditions.

	EUGLYCAEMIC STUDY		HYPOGLYCAEMIC STUDY		
	Baseline	Euglycaemia	Baseline	Hypoglycaemia	
	(mean±SD)	(mean±SD)	(mean±SD)	(mean±SD)	
Inspection Time (ms)	51.5±10.5	51.5±10.4	47.6±8.7	58.3±14.4	p<.05
Change Detection (score)	39.6±4.4	41.45±6.2	39.7±3.6	38.3±5.6	p<.005
Movement Detection (score)	55.5±3.6	56.4±2.6	55.3±3.5	52.7±5.0	p<.005
Contrast Sensitivity (score)	40.6±5.6	41.5±6.2	40.8±6.5	38.2±6.3	p<.005
Visual Acuity-right	6/5	6/6+2	6/6+2	6/6+2	p=ns
Visual Acuity-left	6/6+2	6/6+2	6/6+2	6/6+2	p=ns
Near Point (cm)	10.6±2.3	10.9±2.4	10.9±2.5	11.7±2.7	p=ns
Reading Acuity-right (cm)	11.4±2.4	11.5±2.3	12.0±2.4	11.9±2.5	p=ns
Reading Acuity-left (cm)	11.4±2.4	11.4±1.9	12.2±2.1	12.1±2.6	p=ns
Stereopsis (score)	8.6±1.4	8.7 ±1.0	8.6±1.2	8.7±1.4	p=ns
DSS (score)	45.4±7.0	50.1±7.9	46.2±4.2	42.4±5.3	p<.001
TM-B (secs)	28.2±12.6	25.8±11.6	26.7±8.6	29.9±10.3	p<.05

Figure 3.1.2

Glucose profiles during baseline and study conditions

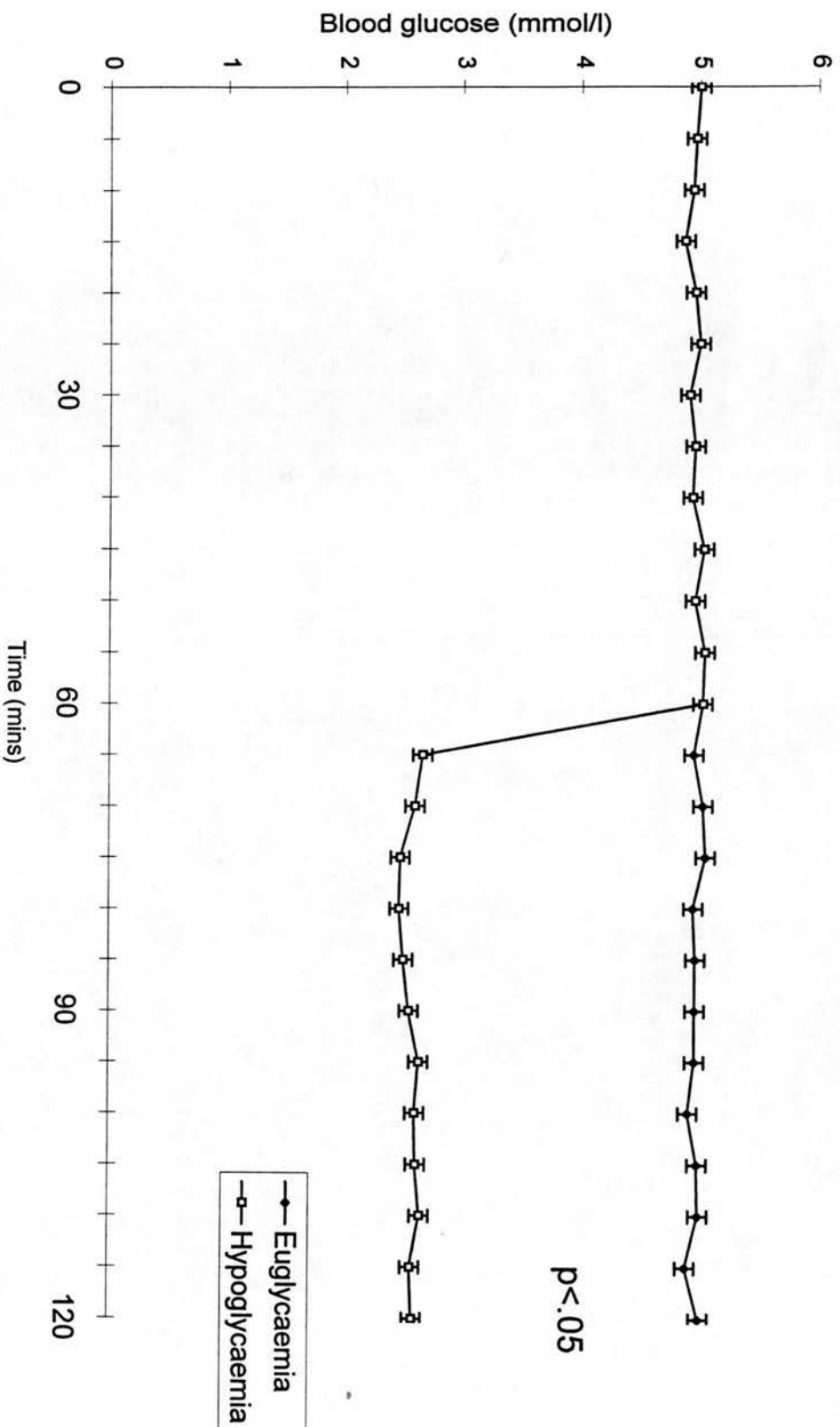


Figure 3.1.3

Contrast Sensitivity measures during baseline(s) and study conditions

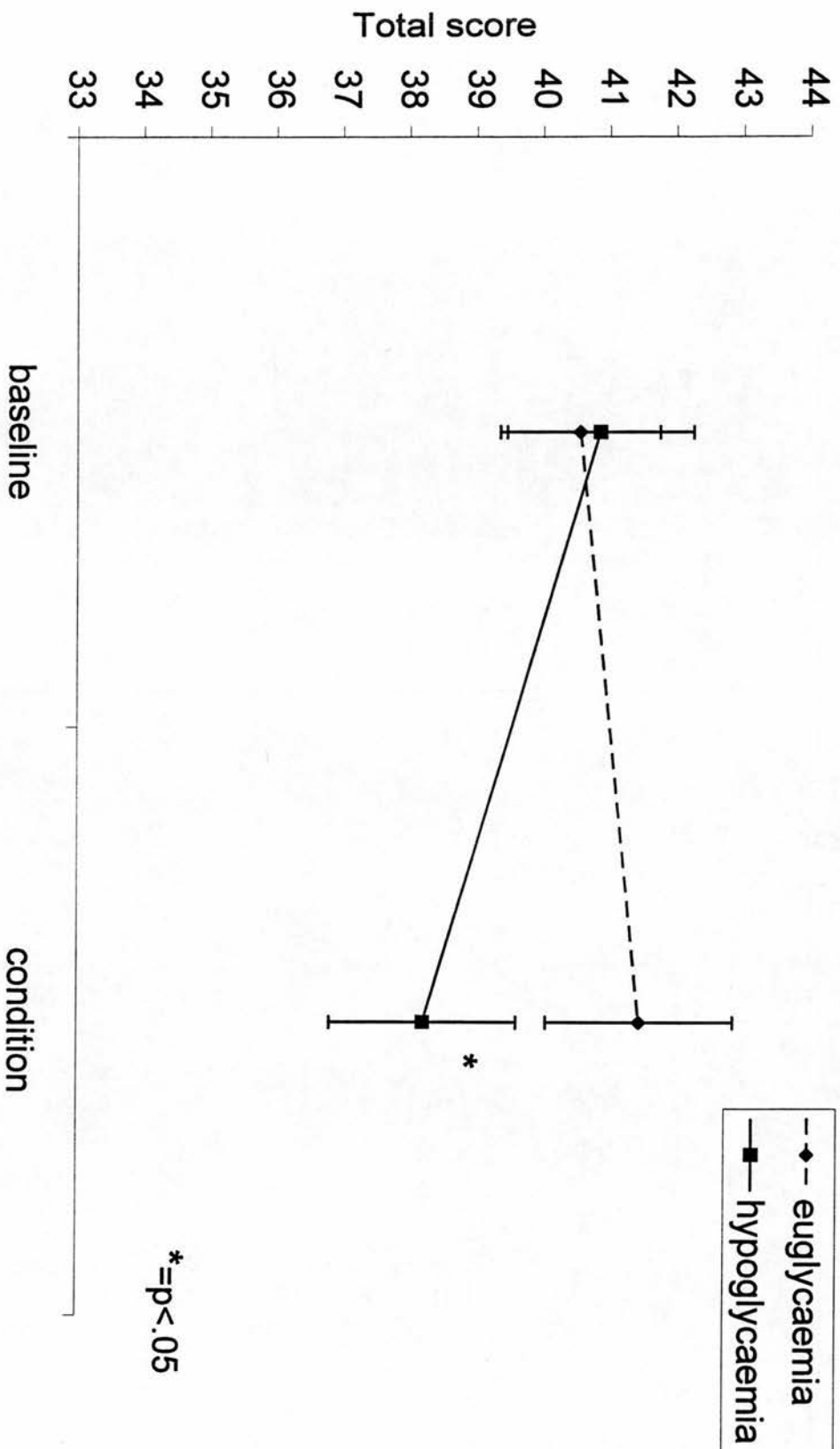


Figure 3.1.4

Performance on the Inspection Time task during baseline and study conditions.

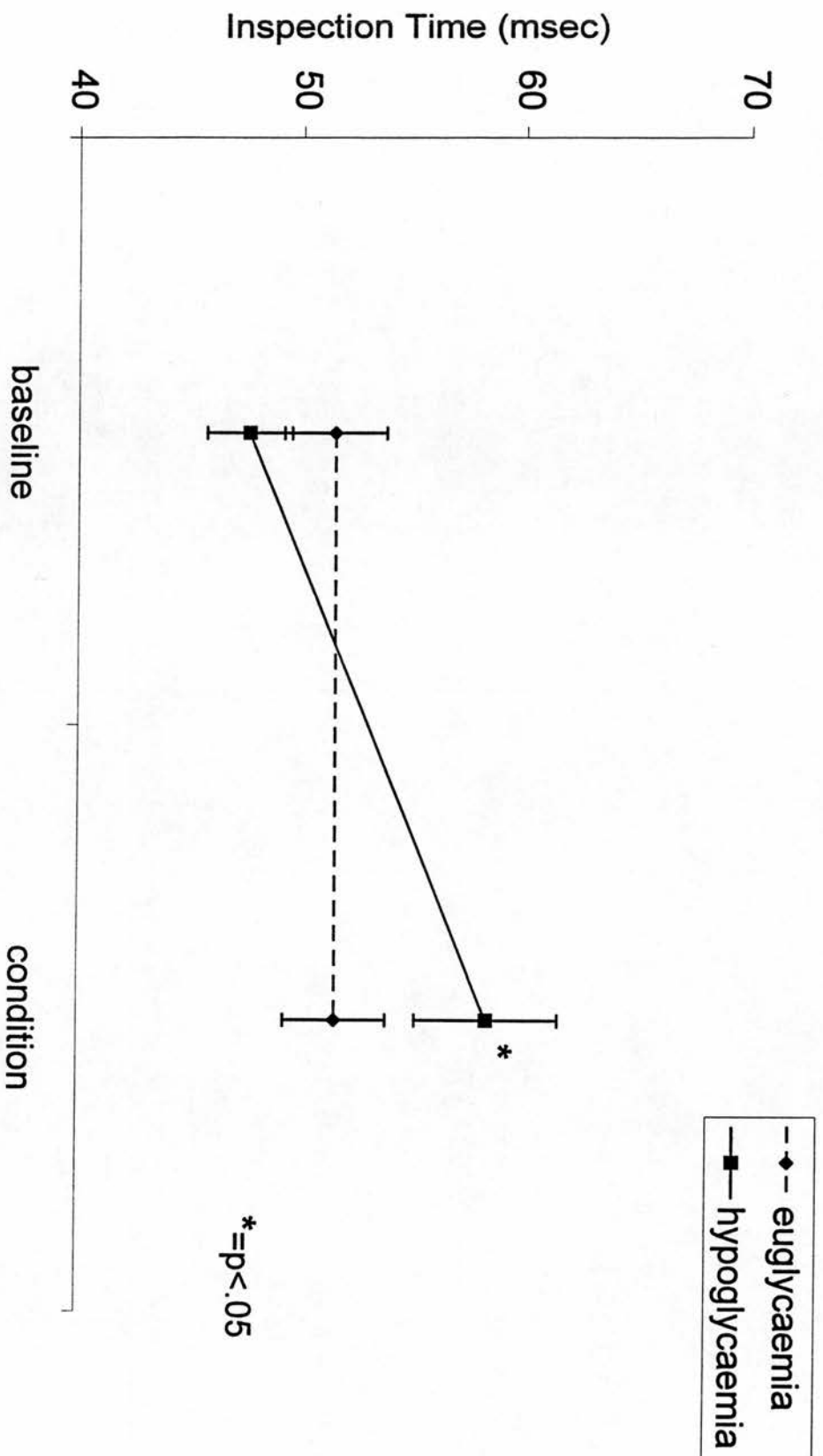


Figure 3.1.5

Performance on Visual Change Detection task during baseline and study conditions

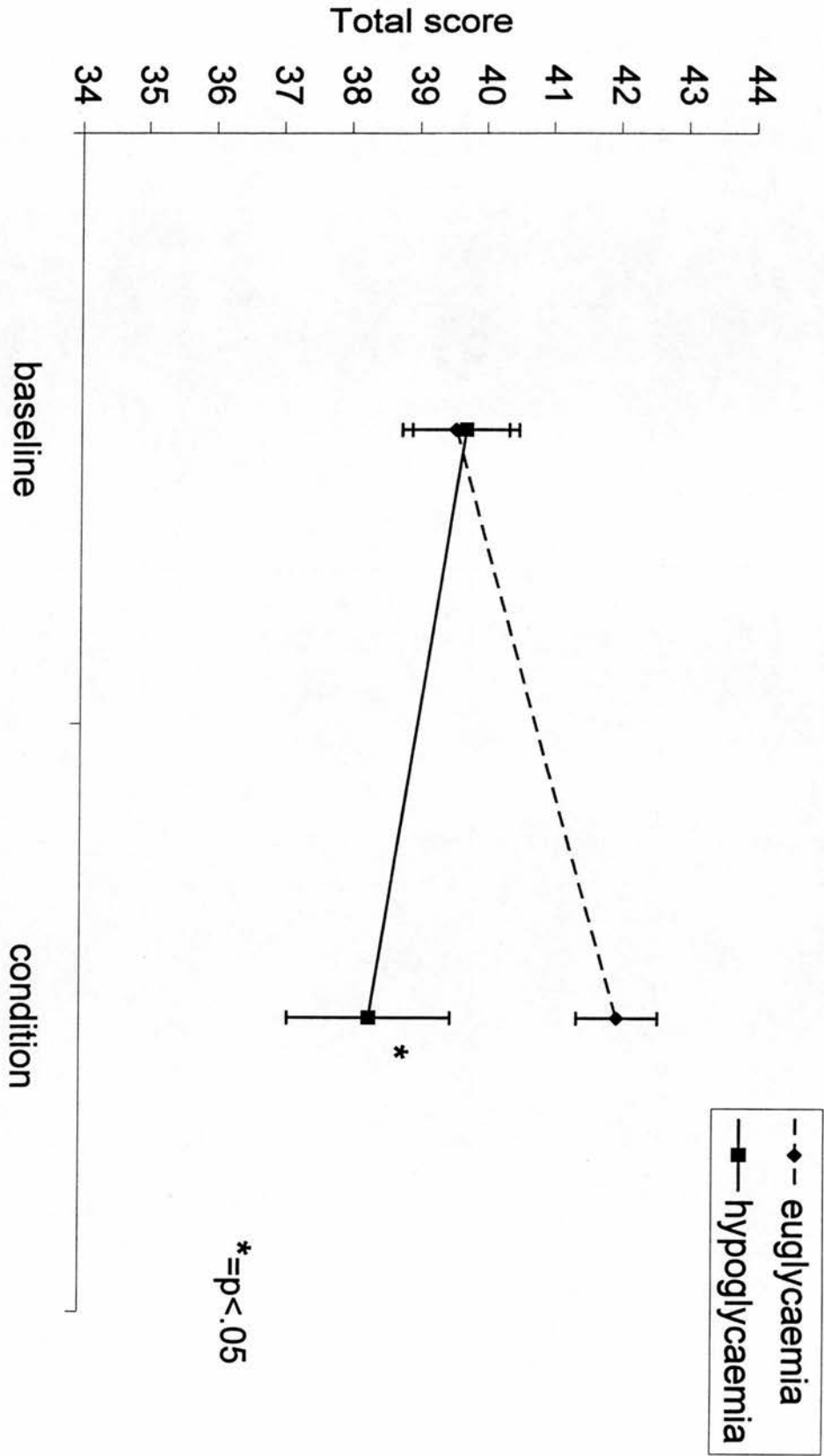


Figure 3.1.6

Performance on Visual Movement Detection task during baseline and study conditions

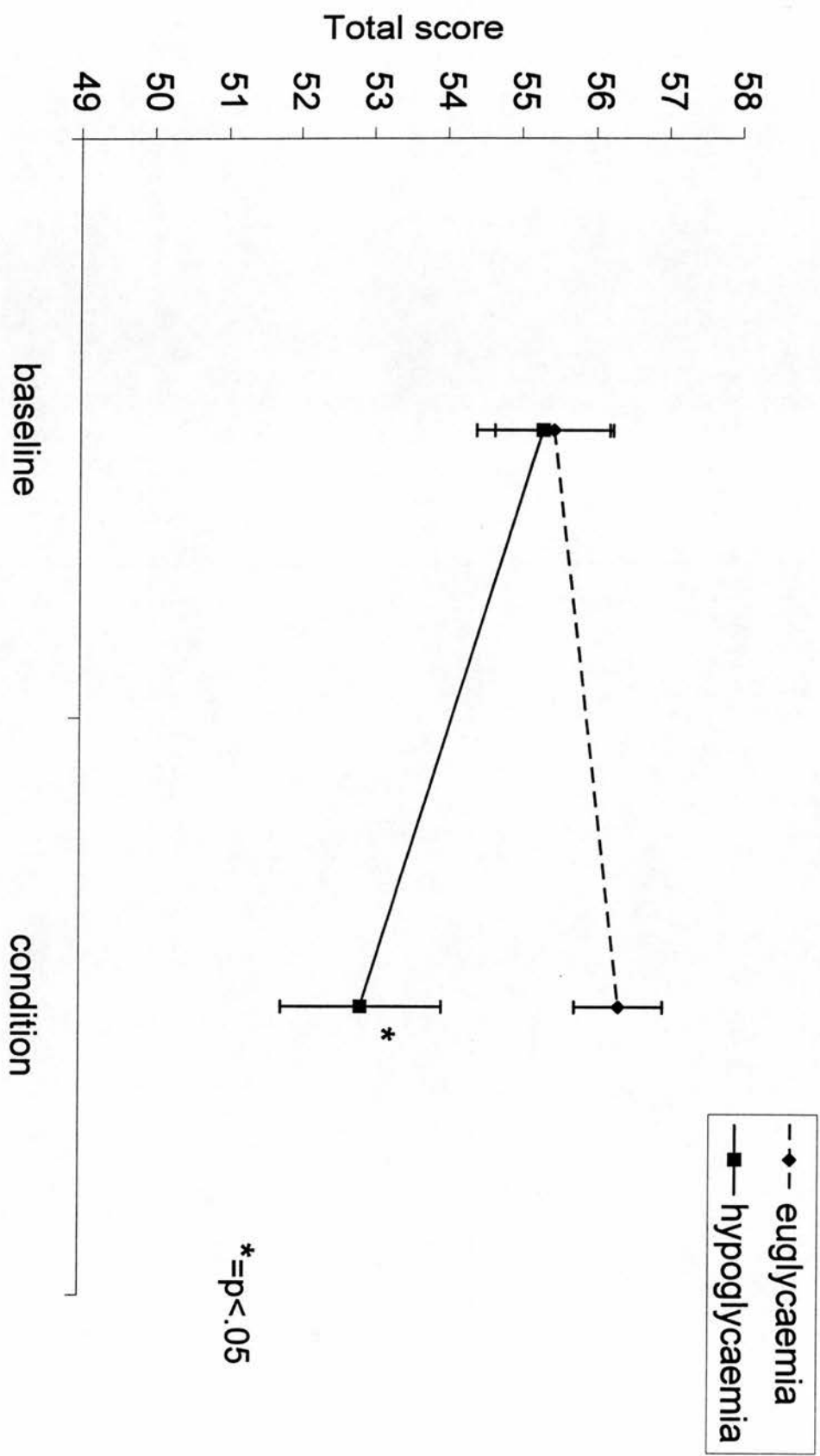


Figure 3.1.7

Stimulus response curves for subject group on the visual change
detection task

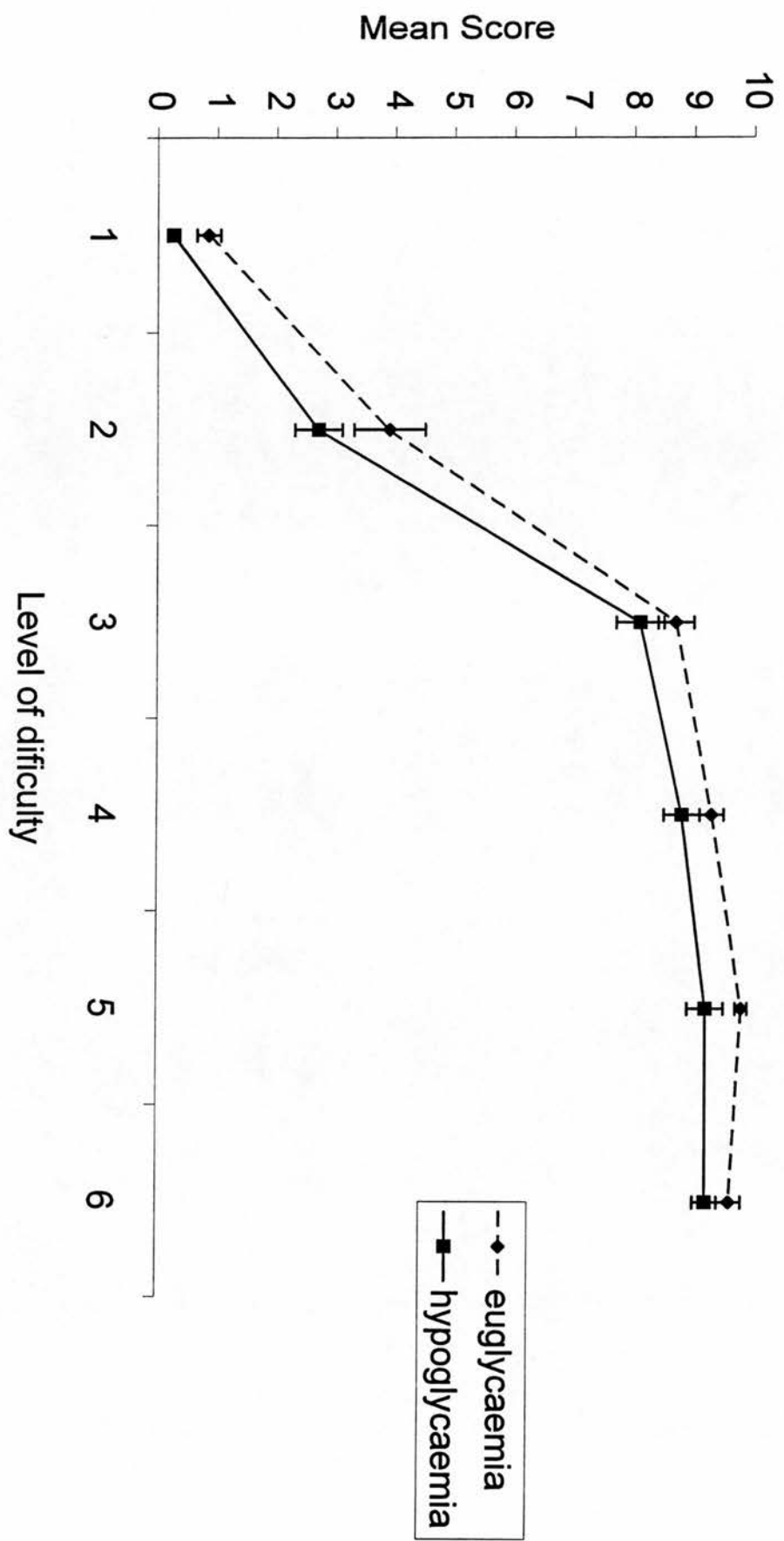


Figure 3.1.8

Stimulus response curves for subject group on the visual movement
detection task

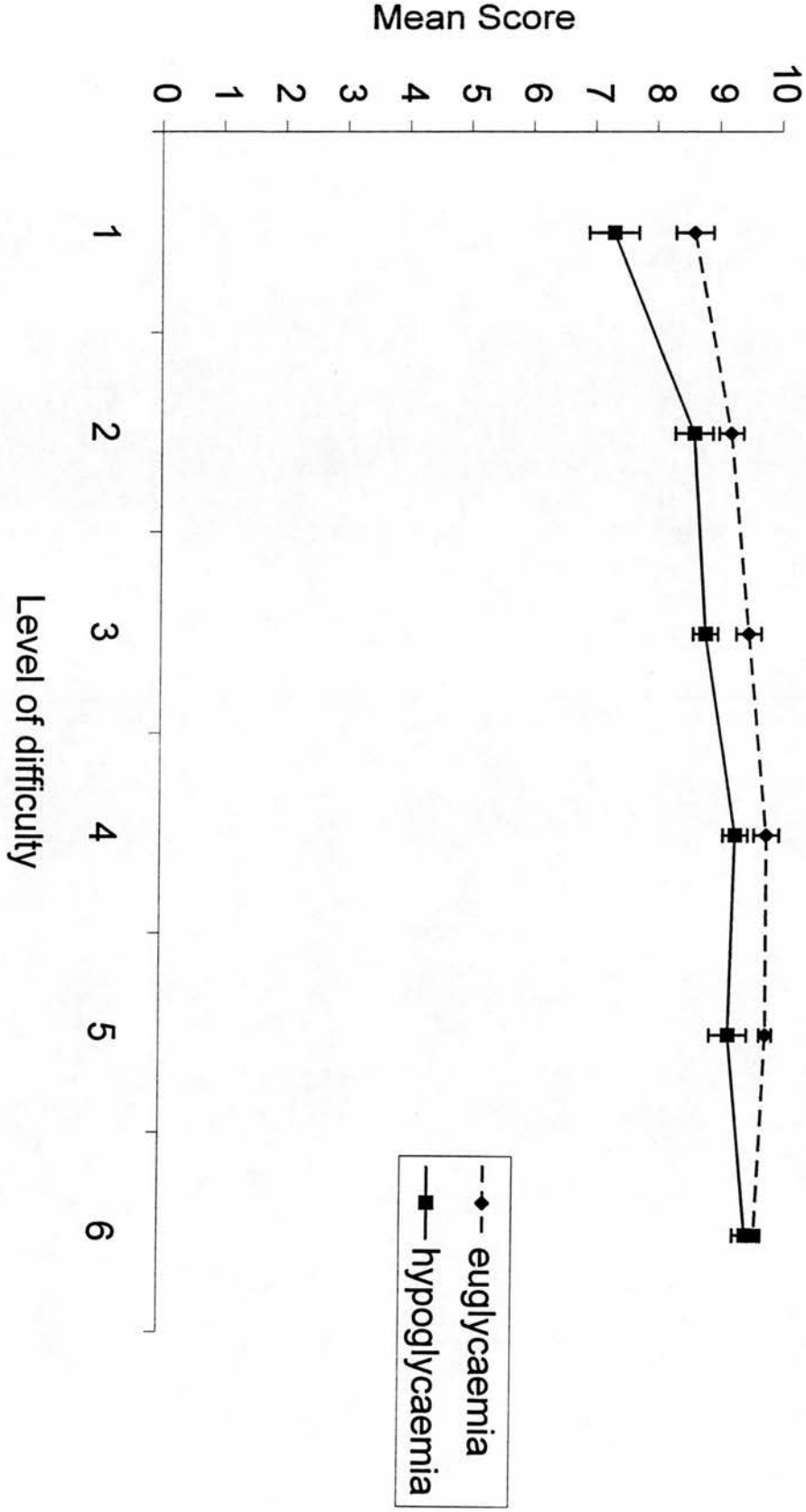
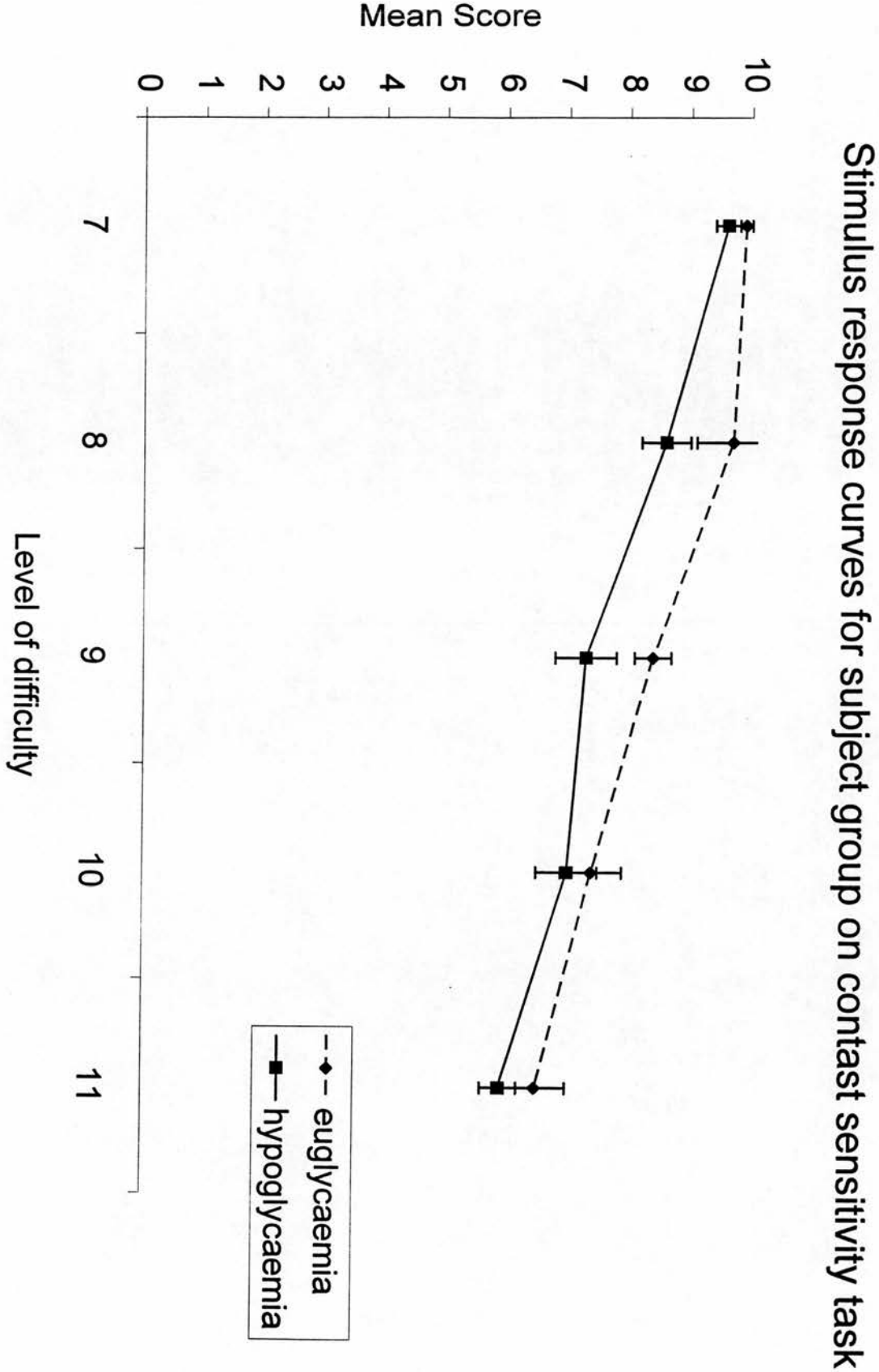


Figure 3.1.9



Discussion

The present study has demonstrated that controlled hypoglycaemia provokes significant changes in visual information processing in a homogeneous group of non-diabetic human subjects. The disruption in visual information processing was accompanied by general cognitive dysfunction. Despite these effects, no changes in standard measures of visual acuity were found during moderate hypoglycaemia. However, visual dysfunction was detected when subjects were required to discriminate between images of low visual contrast.

The response curves for the VCD, VMD and contrast sensitivity tasks (shown in Figures 3.1.7-9) provide additional information on the effect of hypoglycaemia on each of these visual information-processing tasks. In any forced choice task it is essential to separate the willingness of an observer to report weak sensations (his criterion) from his ability to detect them (his sensitivity). Characteristically a change in sensitivity affects the x-positions of a response curve and changes in criterion the steepness of the response curve. Wilkins et al. (1988) has examined this aspect of the contrast sensitivity task in some detail. Rather than 'criterion' change, any flattening of the psychometric curves' steepness in this study could be interpreted as increased noisiness in decision making. Hypoglycaemia has been shown in this study to disrupt cognitive function. It is therefore important to show that hypoglycaemia per se has not lead to a change in the subjects' criterion as this would influence our interpretation of the effects of hypoglycaemia on visual information processing. Examination of the graphs of mean score at each level of difficulty on the VCD, VMD and contrast sensitivity tasks at euglycaemia and hypoglycaemia shows clearly that it is the position of the response curve on the x-axis which has changed as a result of hypoglycaemia and not its steepness. This means that hypoglycaemia has caused a true change in the subjects' sensitivity during each of these visual information-processing tasks.

The interpretation of the information processing tasks used in this study has been derived from experimental psychology and psychophysics. This allows a more detailed parsing of the cognitive dysfunction in hypoglycaemia than would be possible with standard neuropsychological tests. The Inspection time, VCD and VMD tasks are thought to measure the efficiency with which information in iconic (sensory) memory is transferred to discrimination processes in working memory (Deary et al. 1991; Phillips, 1974). If the early information processing tasks described do reflect the time taken to make a single observation from the sensory input, then it also seems likely that this measure will function as a basic factor limiting perceptual and cognitive performance in general (Vickers and Smith, 1986). Consistent with this hypothesis is the correlation between mental processing speed and general mental ability, and the sensitivity of mental processing speed to general cognitive stresses. The present study has clearly demonstrated abnormalities in this important early stage of visual information processing in a homogeneous group of human subjects when the brain is stressed by controlled hypoglycaemia. Hypoglycaemia is also a general cerebral stress and therefore it is possible that the abnormalities in early visual information processing may reflect a more general disruption in information processing. The disruption in this fundamental aspect of cognition may have profound effects on the functioning of the human subject in everyday life who is exposed to hypoglycaemia.

In the present study the information processing tasks have assessed the ability of each subject to discriminate between stimuli within either a narrow (IT) or a broad (VCD and VMD) attentional field. *Both* types of test were significantly disrupted by moderate hypoglycaemia. The IT task is a simple two-choice discrimination task with stimuli presented at differing speeds in a fixed location with no distracters, whereas the VCD and VMD tasks incorporate a changing or moving stimulus, respectively, within a broad field of 49 similar images, whilst also changing the

stimulus presentation speed. It could be argued therefore, that it is information processing speed per se that is disrupted during moderate hypoglycaemia irrespective of the specificity of the attentional demands, or the complexity, of the task.

The present study has also demonstrated a significant deterioration in contrast sensitivity during moderate hypoglycaemia, but no changes in standard measures of visual acuity. These results indicate that low-contrast test targets (where the image is degraded) reveal visual irregularities that are not shown by high-contrast test targets. In Parkinson's disease (Regan and Maxner, 1987) and multiple sclerosis (Regan et al. 1980) abnormalities in contrast sensitivity have also been documented. The disruption in contrast sensitivity in these conditions was orientation selective and, as orientation sensitive neurones are only found in the visual cortex, the authors suggested that these abnormalities might be due to a functional abnormality in the striate cortex as opposed to the retina. In the present study there was a general increase in the total score achieved on the contrast sensitivity task on the euglycaemic study day (Fig.3.1.3). This practice effect also suggests the involvement of higher cognitive processing in the contrast sensitivity task. Whilst there are methodological differences preventing direct comparison of the present study with that of Regan and colleagues (1987) it is possible that the contrast sensitivity task may not distinguish between abnormalities at the retina or of higher cognitive processing.

Our results indicate that visual discrimination for standard clinical acuity tests is unaffected by hypoglycaemia despite a concurrent disruption in cognitive performance tasks. This is consistent with a previous study (Harrad et al. 1985). Harrad *et al.* (1985) did demonstrate abnormalities of colour discrimination (a more sensitive index of macular vision) during hypoglycaemia in a small number of type 1 diabetic and non-diabetic subjects. However, the hypoglycaemia was induced by

intravenous bolus injection of insulin and was uncontrolled and often quite profound. Furthermore, the group of type 1 diabetic subjects all had diabetic retinopathy of various degrees. By contrast, Hardy *et al.* (1995) examined 10 aretinopathic type 1 diabetic subjects during controlled hypoglycaemia (2.5 mmol/l) and found no significant changes in colour discrimination. Hypoglycaemia is a general metabolic stress and it is probable that physiological changes within the retina will influence changes in higher visual pathways and vice versa. In the light of these studies it is therefore important that any assessment of information processing is conducted using high-contrast stimuli to minimise any subtle changes in visual function at the retinal level.

The results of the present study have important practical and theoretical implications. These results imply that if an examination of visual information processing employs images of high contrast that are produced in a *perceptually* unspedded manner then abnormalities of visual processing will not be found during moderate hypoglycaemia. However, if the stimulus is degraded by lowering contrast *and/or* shortening its duration then abnormalities of visual processing will be revealed. The important practical implications relate primarily to the type 1 diabetic patient for whom real life decisions are most often made on the basis of information provided under conditions of low contrast and short perceptual duration.

Abnormalities in driving performance during moderate hypoglycaemia have been demonstrated (Cox *et al.* 1993), and our findings would suggest that these may stem partially from a more basic disruption in information processing. The present findings may also have implications for the employment of type 1 diabetic subjects in other occupations which rely heavily on decision-making based on visual information (e.g. air-traffic control, or commercial pilots, or drivers) who may be exposed to intermittent hypoglycaemia of varying severity.

In summary:-

At a blood glucose level of 2.6 mmol/l significant disruptions in the early stages of visual information processing can be demonstrated in non-diabetic individuals. The disruption in visual information processing is accompanied by evidence of general cognitive dysfunction. However, no changes in standard measures of visual acuity were found during moderate hypoglycaemia although visual dysfunction was detected when subjects were required to discriminate between images of low visual contrast.

PART 3

STUDY 2

Auditory information processing during acute hypoglycaemia

The effect of hypoglycaemia on psychometric aspects of auditory information processing is also largely unexplored. In studies using neurophysiological techniques it has been shown that reversible abnormalities in brain-stem auditory evoked potentials (BAEPs) occur in non-diabetic (Jones et al. 1990) and type 1 diabetic subjects (Lingenfelser et al. 1993a; Ziegler et al. 1991) during hypoglycaemia. Several groups have also shown changes in the auditory P300 wave in response to 'odd-ball' tasks during hypoglycaemia (Jones et al. 1990; Kern et al. 1990; Tallroth et al. 1990; Ziegler et al. 1991), though this has not been a consistent finding (Lindgren et al. 1996).

The Test of Basic Auditory Capabilities (TBAC) (Watson et al. 1982) is a battery of auditory discrimination tests which has been used extensively in auditory research, especially that which has explored the relationships between auditory perception and reading disabilities (Watson and Miller, 1993; Watson, 1992). The TBAC was derived from the examination of a large number of subjects with normal hearing (Watson et al. 1982). Analysis of 22 different tests suggested 8 subtests that best described the major dimensions of auditory capability, and these 8 subtests were grouped into three primary dimensions of auditory ability: simple auditory discrimination, auditory temporal processing (non-verbal) and speech perception (Johnson et al. 1987; Watson and Miller, 1993). The TBAC is also thought to

provide an overall measure of the speed and efficiency of auditory information processing (Watson and Miller, 1993). Performance on the TBAC is unaffected by mid- or high-frequency hearing loss (Christopherson and Humews, 1992). The psychometric properties of the TBAC and its test-retest reliability in normal and in elderly hearing-impaired listeners have been validated (Christopherson and Humews, 1992). The aim of the present study was therefore to examine the effect of controlled hypoglycaemia on basic, validated aspects of auditory information processing in a homogeneous group of non-diabetic subjects.

Methods

Subjects

Twenty (15 male, 5 female) healthy non-diabetic human subjects were studied, who were aged 27 (21 - 42) years (median (range)) and had a Body Mass Index of 23 (19-26) kg/m². All of the subjects had above average intellectual ability as assessed by the National Adult Reading Test and the Alice Heim 4 Test (standard psychometric tests of intellectual ability). None of the subjects had any previous medical history or a family history of diabetes, nor were they taking any regular medication. None of the subjects had a history of hearing difficulties. Subjects were recruited by local advertisement, and were not paid for their participation in the study. Each subject was informed that they would be required to attend the department on three separate occasions. On two of these occasions they would undergo the experimental procedure during which either euglycaemia would be maintained or hypoglycaemia induced. Although subjects knew that they would undergo both study conditions, they were not informed as to which condition was being performed on each study day. The study was approved by the Healthy Volunteer Studies Medical Research Ethics Sub-Committee of the Lothian Medicine and Oncology Ethical Advisory Committee, and written consent was obtained from all of the subjects.

Experimental procedure

All subjects undertook three laboratory sessions, each at least two weeks apart. The initial visit was to familiarise the subjects with the tests that would be used during the experimental conditions. During the familiarisation, subjects completed all of the psychometric tests, the order and duration of which were the same as that of the experimental conditions. Familiarisation was used to help minimise practice effects during the experimental sessions. The results from this session for each individual were discarded. In the two subsequent visits to the laboratory the subjects underwent a hyperinsulinaemic glucose clamp as described in Part II (pp 85-8).

Arterialised whole blood glucose concentration was stabilised initially at 5.0 mmol/l (baseline phase) on both study days. In one study, hypoglycaemia (2.6 mmol/l) was then induced and maintained for one hour, and in the other study the blood glucose concentration was maintained throughout at 5.0 mmol/l (euglycaemia). An interval of 20 minutes was interposed between baseline and the hypoglycaemic or euglycaemic phases to allow achievement of the new glycaemic level. Subjects were not informed of their blood glucose level at any time during the laboratory sessions. During each phase of the study (each baseline, euglycaemia and hypoglycaemia) the subjects were asked to complete the test battery (see below). During each study phase the blood glucose was stabilised for ten minutes at the target glycaemic level before the assessment of information processing. The subjects underwent the experimental conditions in a counterbalanced fashion (i.e. half the subjects underwent hypoglycaemia first followed by the euglycaemic control condition, and half the reverse).

Test battery

During each phase of the study (each baseline, euglycaemia and hypoglycaemia) the subjects underwent tests of:

Auditory information processing tests [TBAC] (Watson et al. 1982).

- 1 Pitch discrimination
- 2 Single-tone loudness
- 3 Single-tone duration
- 4 Temporal order for tones

Psychometric tests of general cognitive function

- 5 Digit Symbol Substitution test [DSS] (Wechsler, 1981).
- 6 Trail Making B test [TM-B] (Reitan and Davison, 1974).

Hypoglycaemia Symptoms

- 7 Edinburgh Hypoglycaemia Scale (Deary et al. 1993b).

The tests used in the battery are described in detail in Part II. Subjects completed the tests in the same order during each study condition, and the battery took between 40-45 minutes to complete.

Statistical analysis

The results were analysed independently for each measure of auditory information processing, test of general cognitive function, and symptom score. A mixed model Analysis of Variance (ANOVA) was used with order of session as a between-subjects factor, with two levels (euglycaemia - hypoglycaemia or hypoglycaemia - euglycaemia), and study condition as a within-subjects factor with two levels (euglycaemia vs. hypoglycaemia). Test results at baseline were used as variable co-variates in each analysis.

Results

Blood glucose

The mean \pm S.D blood glucose during the hypoglycaemia clamp was 2.6 (± 0.2) mmol/l, and 5.0 (± 0.4) mmol/l during the euglycaemia clamp (Fig.3.2.1). The coefficient of variation in blood glucose was 0.6% and 0.7% for the euglycaemic and hypoglycaemic clamps respectively. The initial statistical analysis revealed that no significant order effects (asymmetrical transfer effects) had occurred for any of the outcome variables in this study.

Symptoms

Significant increments in total autonomic symptom scores ($F = 34.4$, $df\ 1, 18$, $p < .001$) and in total neuroglycopenic symptom scores ($F = 7.6$, $df\ 1, 18$, $p < .05$) were recorded during the hypoglycaemia condition of the study (Table.3.2.1). No significant change occurred in symptoms of hypoglycaemia-associated malaise ($F = .25$, $df\ 1, 18$, $p = ns$).

General cognitive function

In the present study scores achieved on the DSS were significantly lower ($F = 31.0$, $df\ 1, 18$, $p < .001$), and times taken to complete the TM-B were significantly longer ($F = 9.8$, $df\ 1, 18$, $p < .01$) during hypoglycaemia when compared with euglycaemia (Table.3.2.1). This confirmed that these standard measures of general cognitive function were significantly affected at a blood glucose of 2.6 mmol/l.

Auditory information processing

The TBAC is designed to yield overall percent-scores of 75-90% in young adults with normal hearing (Christopherson and Humews, 1992). It is recognised that within the TBAC there will be intra- and inter-individual variation in performance

on the tests. This is consistent with the existence of independent dimensions of auditory capability (Johnson et al. 1987). In the present study subjects' overall percent-correct scores (achieved by adding total scores for each subtest to provide an overall percentage score for the test battery for each individual) on each of the study days at baseline were: 85% (76-94%) (euglycaemia study), and 85% (75-93%) (hypoglycaemia study); Spearman correlation = .86 ($p < 0.01$). Individual performances therefore, were stable across time, and consistent with previous studies (Christopherson and Humews, 1992; Johnson et al. 1987).

Acute insulin-induced hypoglycaemia caused a significant deterioration in auditory temporal processing, and in one of the three simple auditory processing tasks (Table.3.2.1, Fig.3.2.2-5). The ability of the subject group to discriminate the temporal order of two tones was impaired during moderate hypoglycaemia ($F = 9.91$, df 1, 18, $p < .01$) (Fig.3.2.2). Hypoglycaemia also provoked an impairment in ability to discriminate the loudness of a single tone ($F = 6.1$, df 1, 18, $p < .05$) (Fig.3.2.3). No significant change was noted in the subjects' ability to detect the duration ($F = 1.7$, df 1, 18, $p = ns$) or pitch ($F = .64$, df 1, 18, $p = ns$) of a single tone (Fig.3.2.4 and Fig.3.2.5).

Table3.2.1. Measures of auditory information processing, general cognitive performance, and hypoglycaemia symptoms during each study condition.

	EUGLYCAEMIC STUDY		HYPOGLYCAEMIC STUDY		
	Baseline	Euglycaemia	Baseline	Hypoglycaemia	
Temporal Order (score)	55.4 (7.5)	56.7 (6.5)	54.8 (7.9)	52.1 (7.7)	p<0.01
Pitch Discrimination (score)	63.5 (4.6)	63.7 (3.9)	61.9 (5.1)	61.3 (4.8)	p=ns
Single-tone Loudness (score)	65.9 (3.9)	64.6 (4.5)	66.5 (4.4)	62.7 (5.5)	p<.05
Single-tone Duration (score)	59.9 (4.5)	59.3 (4.9)	61.1 (3.9)	57.3 (5.7)	p=ns
Digit Symbol (score)	69.9 (14.0)	73.2 (14.6)	68.9 (14.8)	66.1 (12.5)	p<.001
Trail Making B (secs)	30.5 (13.4)	28.2 (14.4)	31.4 (15.7)	35.6 (16.2)	P<.01
Autonomic symptoms (max score; 28)	8.5 (2.6)	7.8 (2.0)	8.4 (3.6)	14.2 (6.5)	P<.001
Neuroglycopenic symptoms (max score; 35)	8.2 (3.4)	8.0 (5.0)	8.1 (4.7)	10.6 (5.7)	p<.05

All values are given as mean (S.D).

Figure 3.2.1.

Glucose profiles during baseline and study conditions

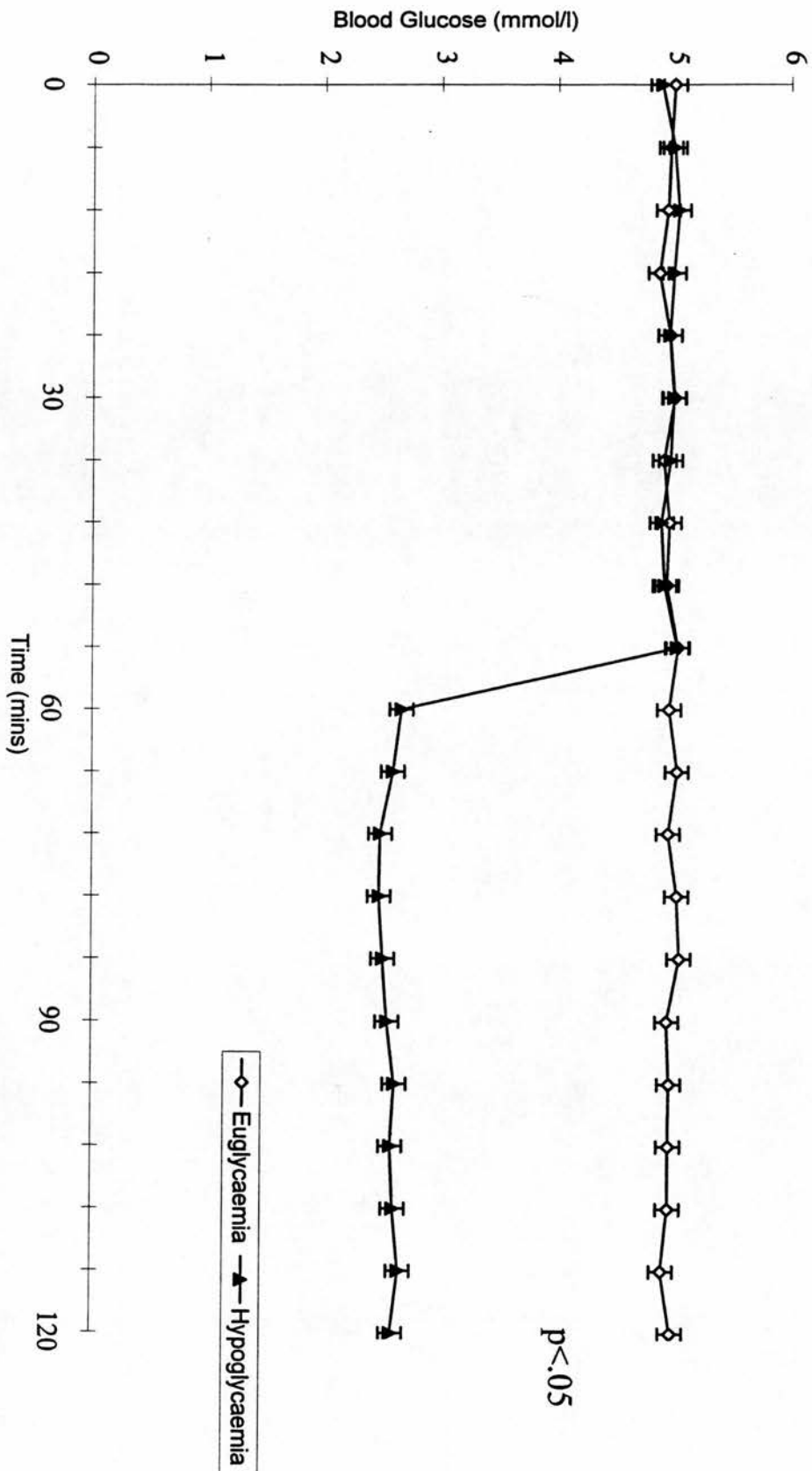


Figure 3.2.2

Temporal-order discrimination during baseline and study conditions

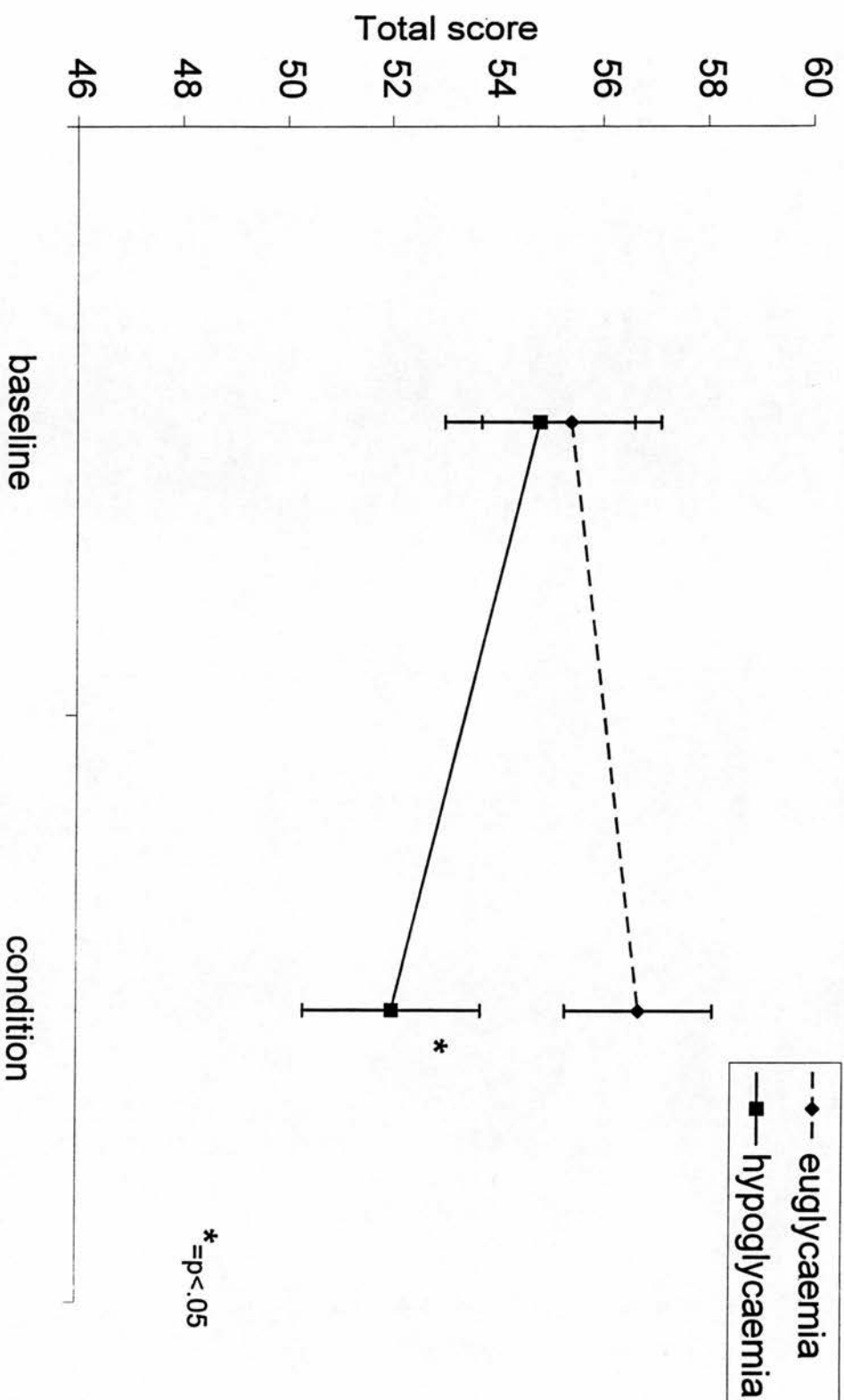


Figure 3.2.3

Single-tone loudness discrimination during baseline and study conditions

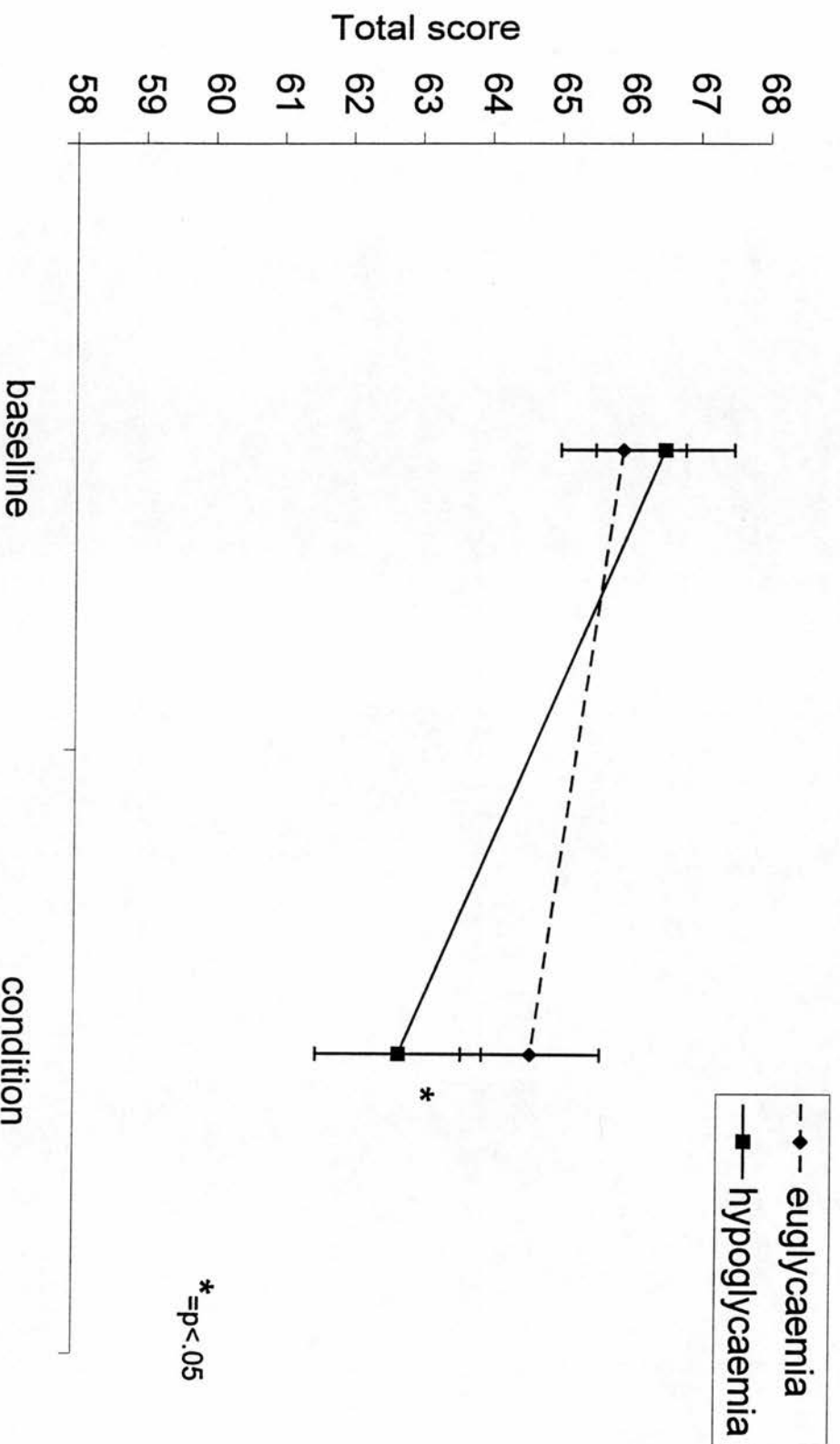


Figure 3.2.4

Single-tone duration discrimination during baseline and study conditions

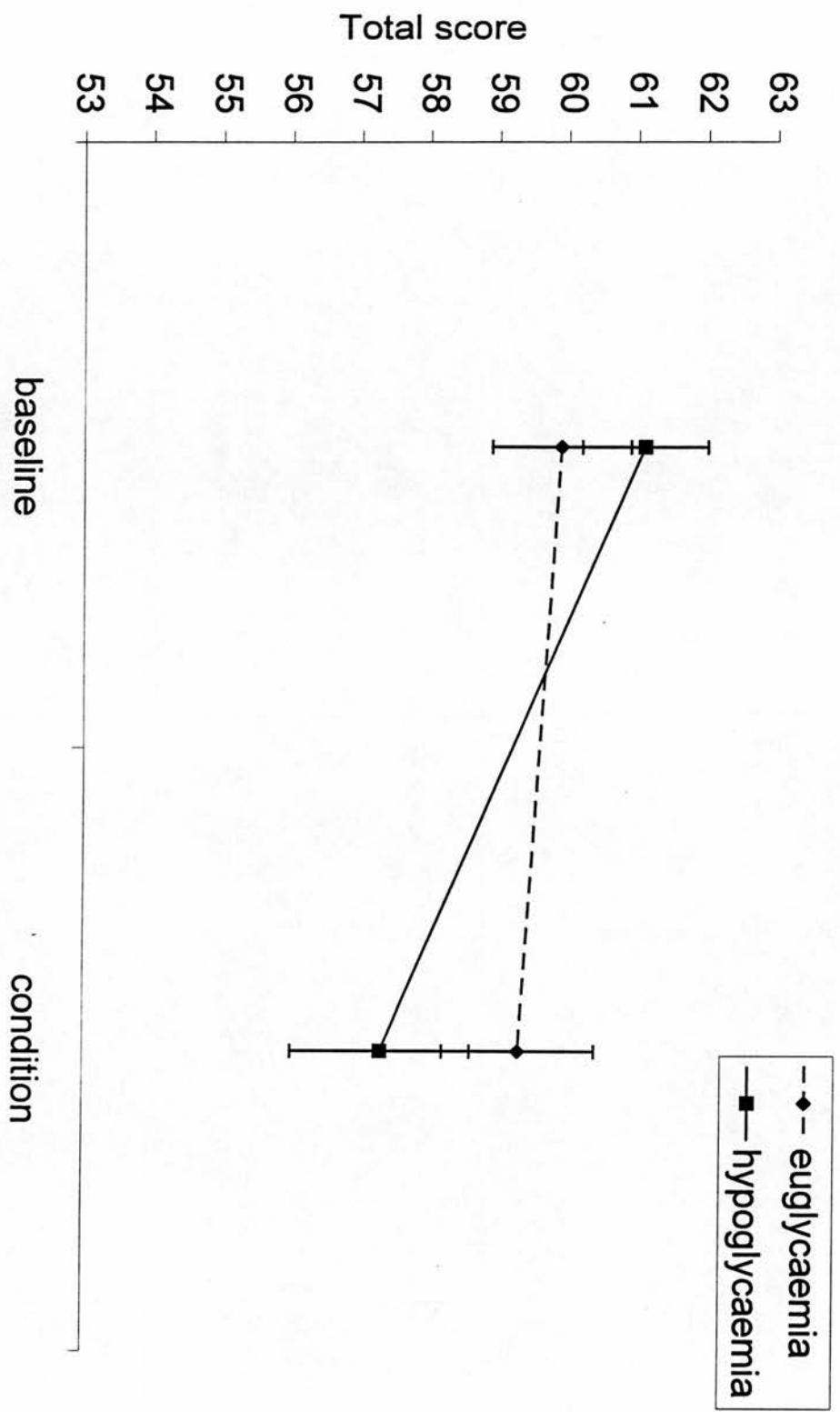
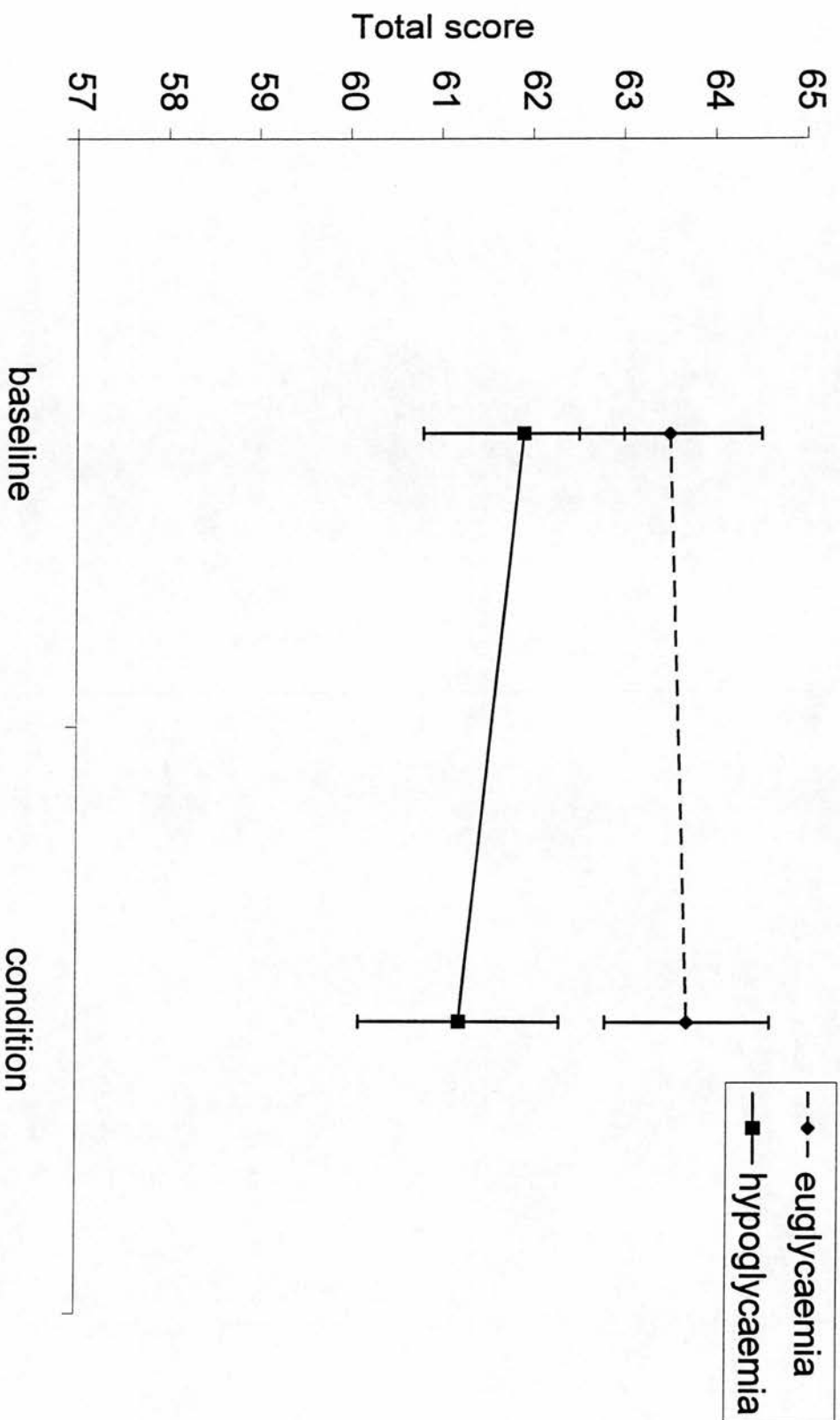


Figure 3.2.5

Pitch discrimination during baseline and study conditions



Discussion

The present study has demonstrated that moderate hypoglycaemia results in a deterioration in specific aspects of basic auditory information processing in a homogeneous group of non-diabetic human subjects. Impairments in auditory temporal processing (non-verbal; temporal order discrimination), and in one of three tests of simple auditory processing (single-tone loudness) have been demonstrated. The impairment in auditory information processing was accompanied by a general cognitive dysfunction. This would suggest that the central nervous system effects of moderate hypoglycaemia are not limited to higher cortical areas and complex cognitive tasks, but extend to more basic information processing functions.

One prominent account of auditory information processing considers there to be two different types of auditory (or echoic) sensory memory; (a) an unanalysed short auditory storage, and (b) a separate, longer memory of auditory information (Cowan, 1984). Short auditory storage refers to the initial recognition of the auditory stimulus, prior to further analysis and retention. The auditory stimulus is therefore experienced as a sensation rather than a memory. Experiments in psychophysics have determined that short auditory storage applies to stimuli of up to 350msec in duration (the TBAC uses tones of up to 250 msec). The TBAC is a measure of short auditory storage and as such examines one specific aspect of basic auditory information processing. The TBAC also utilises information processing tasks, which by manipulating stimulus duration, arguably provide an index of the speed at which individuals are able to process auditory input. The present study therefore reveals two important findings: (i) significant disruptions in short auditory storage during acute insulin-induced hypoglycaemia, and (ii) that hypoglycaemia might slow the speed at which the brain is able to process information provided by hearing.

The present study uses tasks that are arguably very similar in nature to those used in the previous study on the effect of acute hypoglycaemia on visual information processing. In both studies the tasks used are information processing tasks which index the stimulus duration required to make a simple discrimination from the sensory input. Neither study involved speeded responses; in all the tasks discussed the stimulus durations were manipulated and the correctness of the subjects' decisions noted. It is possible that these tasks may be accessing, in addition to any modality-specific performance, a sensory speed or efficiency factor which limits perceptual and cognitive performance in general. There is a considerable body of research on the relationships between sensory processing measures and performance on higher cognitive ability tests, such as IQ-type tests. Significant correlations have been found between traditional psychometric tests of intelligence and measures of visual inspection time, simple and choice reaction time, speed of scanning information in short term memory, speed of retrieval on information from long-term memory, and pitch discrimination (Deary, 1994a; Eysenck, 1988; Vernon, 1983). Furthermore, correlations between auditory information processing and mental ability are also well recognised in the scientific literature (Deary, 1994a; Deary, 1994b; Deary, 1995; Raz et al. 1983; Raz et al. 1987), and have been demonstrated for overall-percent scores on the TBAC (Watson, 1992). Deary (Deary et al. 1991) conducted a longitudinal study that suggested that efficiency of lower level auditory processing is causal to later high-level cognitive ability differences. In a study by Watson (1992), scores achieved on each of the 4 sub-tests of the TBAC used in the present study correlated significantly with scores achieved on the mathematical section of the Scholastic Aptitude Test (SAT). The importance of this research for the present study is that individual deficits in low-level cognitive processing may have widespread effects on cognitive performance.

Hypoglycaemia may be exerting its primary effect on other regions of the auditory pathway such as the cochlear or auditory nerve. However, as far as we are aware

there are no studies which show an effect of hypoglycaemia on standard measures of hearing and those studies that have assessed neurophysiological aspects of the auditory pathway have tended to show abnormalities in 'central' processing. Ziegler and colleagues (1991) found that there was an increase in the inter-peak latency of waves III - V and latency of wave V at a blood glucose level of 1.7 mmol/l. The latency of wave I was unaffected. The authors suggested that this provided evidence that hypoglycaemia induces a conduction delay in the central segments of the brain stem (superior olivary complex to inferior colliculus) without auditory nerve impairment (Ziegler et al. 1991). Jones et al. (1990) supported this finding when they reported a decrease in the amplitude of wave V of BAEP in a small group of non-diabetic subjects during hypoglycaemia (arterialised blood glucose 3.0 mmol/l). This group have also demonstrated in an elegant experiment, using microelectrodes implanted in the rat brain, that the peak V wave of the BAEP reflects activation of the Inferior Colliculus, and that the predominant effect of hypoglycaemia appears to occur in or near the Inferior Colliculus in this animal model (Weber et al. 1994). Abnormalities in the auditory P300 wave have also been demonstrated both in type 1 diabetic and non-diabetic subjects during hypoglycaemia (Jones et al. 1990; Kern et al. 1990; Tallroth et al. 1990).

Individual differences in the waveforms of event-related potentials (P200) have been shown to correlate with performance on visual and auditory inspection time, and pitch discrimination tasks (Caryl et al. 1995). Caryl and Harper (1996) demonstrated, in a group of 35 undergraduate students, that differences in thresholds and accuracy of responding on a visual inspection and a pitch discrimination task were reflected in differences in the waveforms of event-related potentials (latency and amplitude of the p300 wave). These two groups of evidence (the effect of hypoglycaemia on neurophysiological measures and the relationship between psychometric measures of information processing and event-related potentials), and the finding of the present study that hypoglycaemia has an effect on psychometric

measures of information processing (i.e. that both psychometric tests and neurophysiological measures point to the same sort of variance) indicate that both techniques should be incorporated in future study design to more clearly describe the effects of hypoglycaemia on basic sensory processing.

In the present study hypoglycaemia led to a disruption in performance on only one of the 'simple' auditory discrimination tasks [though there is a tendency towards a poorer performance on the single-tone duration task during hypoglycaemia (Fig.3.2.4)]. This finding may reflect differences in threshold amongst these auditory performance tasks during hypoglycaemia. It is recognised that different cognitive performance tasks often show different thresholds for disruption during hypoglycaemia, though this finding has to date been limited to tasks which are non-specific in nature (Deary, 1993). It is possible that hypoglycaemia of a slightly greater degree would have had a more widespread effect on of auditory information processing.

The results of the present study have theoretical and practical implications. Further evidence is provided about the importance of sensory processing speed to perceptual and cognitive performance in general, and of its sensitivity to mild cerebral stresses. The important practical implications relate primarily to the type 1 diabetic person who is frequently exposed to hypoglycaemia of varying severity as a side-effect of treatment. The demonstration of significant, though small changes in aspects of auditory discrimination may have implications for those type 1 diabetic individuals who are involved in such tasks as sonar operation, music, and any task requiring language skills.

In summary:-

At a blood glucose level of 2.6 mmol/l significant disruptions in some aspects of the early stages of auditory information processing can be demonstrated in non-diabetic individuals. This specific disruption in auditory information processing is accompanied by evidence of general cognitive dysfunction.

PART III

STUDY 3

Anger-state during acute insulin-induced hypoglycaemia

The literature on the effects of hypoglycaemia on mood and other non-cognitive aspects of behaviour in human subjects is sparse (Gold et al. 1997). This information is of considerable practical as well as theoretical importance. Negative mood states and behavioural change can have an adverse effect upon an individual's social and work relationships, and may explain why the achievement of good glycaemic control is so difficult in some individuals with type 1 diabetes.

The relationship between mood and blood glucose concentrations is idiosyncratic (Gonder-Frederick et al. 1989). However, recent work has demonstrated that certain characteristic mood changes occur during experimental hypoglycaemia in both the non-diabetic (Gold et al. 1997; Gold et al. 1995c; Hepburn et al. 1995) and type 1 diabetic subjects (Weinger et al. 1995; Hepburn et al. 1995). Hypoglycaemia results, in general, in the expression of negative mood states (Gold et al. 1997; Hepburn et al. 1996; Hepburn et al. 1995; Gold et al. 1995c; Gonder-Frederick et al. 1989). The negative mood-state *anger*, as an expression of hypoglycaemia, is recognised, but equally poorly studied. There are reports of irrational and aggressive behaviour in insulin-treated subjects during hypoglycaemia (Adlersberg and Dolger, 1938; Maher and Frier, 1993). In the 1970's there was considerable interest in the relationship between 'reactive hypoglycaemia' and aggression. It was proposed that certain individuals responded to the sugar content in a meal by the overproduction of insulin

to such an extent that several hours after a meal 'rebound hypoglycaemia' occurred. Some investigators found that reactive hypoglycaemia occurred with a greater frequency in subjects with a violent background (Bolton, 1973; Bolton, 1976; Virkkunen, 1982; Virkkunen, 1986; Virkkunen and Huttenen, 1982). Benton and colleagues (1982) gave a glucose tolerance test to a group of males with no past history of violent and anti-social behaviour, and observed a relationship between the tendency of the blood glucose to fall and scores on several questionnaire measures of aggression. The relevance of these findings has been questioned because, as Benton (1988) himself has pointed out, the glucose tolerance test is an unphysiological technique which creates a model for hypoglycaemia that will rarely be encountered by most normal individuals. Studies using the oral glucose tolerance test are also complicated by the fact that individuals are subjected to different absolute levels of blood glucose (a measure of the *rate of change* in blood glucose has been used in most studies as a means to compare individuals rather than the nadir of blood glucose), and the glucose concentration is estimated from capillary blood which does not provide a true reflection of the blood glucose to which the brain is being exposed. Furthermore, as no measures of the classical symptoms of hypoglycaemia (Deary et al. 1993b) were taken, it is difficult to ascertain how many of the subjects were actually 'experiencing' hypoglycaemia. In addition, in all of the studies, except that by Benton and colleagues (1982), a relationship between glucose tolerance test abnormalities and a *previous* history of violent or antisocial behaviour was observed, rather than with measures of aggression taken during the test itself. However, Benton (1988) also argued that, as all of the studies had been very consistent in their findings, they should not be dismissed prematurely, and that "studies should be carried out in which the blood glucose is manipulated and aggression monitored". That is what is done in the present study.

The aim of the present study was to examine the effect of insulin-induced hypoglycaemia on anger-state in non-diabetic human subjects. Anger-state, -trait,

and -expression were assessed using a well validated questionnaire for which normative data are available for more than 9000 subjects (Spielberger, 1991).

Methods

Subjects

A total of 18 subjects were studied: 18 (14 male, 4 female) healthy non-diabetic human subjects of mean (SD) age 27 (8) years. Subjects were recruited by local advertisement, and were not paid for their participation in the study. The study was approved by the Healthy Volunteer Studies Medical Research Ethics Sub-Committee of the Lothian Medicine and Oncology Ethical Advisory Committee. Written consent was obtained from all of the subjects.

Study design

Subjects were asked to attend the laboratory three times, each at least two weeks apart. During the initial visit subjects completed a questionnaire that assessed anger-trait and anger expression (Part II; pp 96-97). In the two subsequent visits to the laboratory the subjects underwent a modified hyperinsulinaemic glucose clamp procedure (DeFronzo et al. 1979). In one condition, hypoglycaemia (2.6 mmol/l) was induced and maintained for one hour, and in the other condition the blood glucose concentration was maintained throughout at 5.0 mmol/l (euglycaemia). During the two study conditions (euglycaemia and hypoglycaemia) subjects completed a questionnaire which assessed anger-state, and a questionnaire assessing symptoms of hypoglycaemia. Subjects were not informed of their blood glucose level at any time during the laboratory sessions, and the order of study conditions was counterbalanced.

Procedure

The details of the hyperinsulinaemic glucose clamp technique are given in Part II (pp 85-88).

Test Battery:

(a) Measures taken prior to clamp studies:

Anger-Trait (Spielberger, 1991)

Anger-Expression (Spielberger, 1991)

(b) Measures taken during clamp studies:

Anger-State (Spielberger, 1991)

Hypoglycaemia Symptoms - Edinburgh Hypoglycaemia Scale (Deary et al. 1993b).

Statistical analysis

Results are expressed as median (range) or as mean (\pm S.D.), as appropriate. A non-parametric Mann-Whitney U test was used initially to examine for order effects. No order effects were noted for any of the variables in the study. The Wilcoxon matched pairs test was used to compare state measures during each condition (hypoglycaemia and euglycaemia). Correlation analyses were performed using Pearson's Correlation or Kendall's *tau-b* as appropriate. Statistical analysis was carried out using the SPSS for Windows statistical package (SPSS Inc, Chicago, Ill, USA).

Results

Stable blood glucose plateau's were achieved during each study. The mean (\pm S.D) blood glucose during the hypoglycaemic clamp was 2.6 (\pm 0.2) mmol/l, and 5.0 (\pm 0.4) mmol/l during the euglycaemic clamp.

Symptom scores for the subject group as a whole increased significantly as predicted during hypoglycaemia. The mean (SD) autonomic symptom score during the euglycaemic condition was 11 (4) and during the hypoglycaemic condition was 19 (10) ($p < 0.05$). Similarly, the mean (SD) neuroglycopenic symptom score during the euglycaemic condition was 14 (9) and during the hypoglycaemic condition was 20 (13) ($p < 0.05$). (The lowest possible autonomic and neuroglycopenic symptom scores were 4 and 5 respectively).

Group mean and standard deviation scores on each of the measures of anger-trait and anger-expression (Table.3.3.1) fall within the expected normal range for this age group (Spielberger, 1991).

Anger-state scores during euglycaemic and hypoglycaemic conditions are shown in Table 3.3.1. There was an increase in reported anger-state from a median (range) score of 10 (10-13) during euglycaemia to 11(10-27) during hypoglycaemia. This increase in anger-state was significant: $z = -2.9$; $p < 0.005$.

To explore possible relationships underlying the observed effect of hypoglycaemia on anger-state, a correlational analysis was performed between the residuals obtained from regressing anger-state scores reported during hypoglycaemia against those reported during euglycaemia and the separate measures of anger-trait and anger-expression. Because of the number of tied ranks and relatively small n ,

Kendall's *tau-b* was chosen. No significant correlation's were found between the residuals obtained and the measures of anger-trait and anger-expression.

A final correlational analysis was performed between an individual's change in anger-state, and the change in autonomic and neuroglycopenic symptom scores between the two study conditions. The correlation's between the change in anger-state and change in autonomic symptoms and neuroglycopenic symptoms were: $r = 0.13$ ($p=0.39$) and $r = 0.23$ ($p=0.12$) respectively. However, the correlation between autonomic and neuroglycopenic symptom change was highly significant: $r = 0.77$, $p<0.001$. This implies that the change in anger-state showed no significant association with an individual's symptomatic experience of hypoglycaemia.

Table 3.3.1. Measures of Anger state, trait, and expression in Non-diabetic subjects.

	Non-Diabetic Group	Normative Data
	Median (range)	Mean (S.D)
<u>Anger-state</u>		
euglycaemia	10 (10-13)	11.3 (3.2)
hypoglycaemia	11 (10-27)*	
<u>Anger-expression</u>		
AX /Con	23 (17-27)	26.2 (4.3)
AX /Ex	24 (16-37)	19.4 (7.4)
AX /In	16 (11-22)	15.4 (3.9)
AX /Out	14 (12-24)	14.4 (3.3)
<u>Anger-trait</u>		
T-ang	19 (15-24)	18.7 (4.8)
T-ang-R	10 (7-13)	9.3 (2.6)
T-ang-T	4 (4-12)	6.2 (2.5)

Reported measures of anger-state during study conditions, measures of anger-expression and anger-trait taken during familiarisation session, and normative data for the STAXI (normal male values are given because most of the subjects in this study were male but they do not differ substantially from normal females). * = $p < 0.05$ hypoglycaemia vs. euglycaemia.

Discussion

This is the first study to examine the effects of hypoglycaemia on anger-state under controlled experimental conditions using a validated measure of anger-state.

Hypoglycaemia has been shown to induce an overall increase in anger-state in non-diabetic individuals under stable laboratory conditions. The change in anger-state could not be predicted from measures of anger-trait or anger-expression, nor did it relate to the intensity of the symptomatic experience of hypoglycaemia.

The findings of this study lend weight to the argument that the relationship between hypoglycaemia and anger has been prematurely dismissed (Benton, 1988). The problem that had been encountered in the interpretation of studies such as that by Benton et al. (1982) is that the use of the glucose tolerance test can lead only to the finding of a correlation between a non-physiological test and a questionnaire measure of aggression. The intrinsic difficulties of interpreting a correlational analysis are well known. Using the hyperinsulinaemic glucose clamp technique enables one to manipulate the blood glucose in a controlled manner whilst at the same time measuring individual responses in terms of anger experienced during this altered physiological state. A small, but significant, increase in median anger-state score during moderate hypoglycaemia was demonstrated. Although the increase in anger-state was small it is important to note that the study was carried out in a non-confrontational laboratory setting that would be unlikely to stimulate the expression of anger in the form of aggressive behaviour. Despite this, a marked increase in state-anger was observed in some individuals, and a slight increase in most others. It is possible that the increase in anger shown by some individuals during hypoglycaemia may lead to aggressive behaviour depending on provocation or other aspects of the situation. The present studies findings may provide some explanation for those rare circumstance where hypoglycaemia has occasionally resulted in very

aggressive behaviour (Maher and Frier, 1993). The few subjects who had large rises in anger during hypoglycaemia are of particular interest, and this type of individual warrants further study.

It remains possible that the relationship between blood glucose levels and aggressive behaviour share a common mechanism but that they are not causally related. For instance, individuals who experience a more profound physiological stimulus during hypoglycaemia may be more likely to show a change in anger-state (i.e. those individuals who experience more intense autonomic symptoms [reflecting the catecholamine response to hypoglycaemia] or neuroglycopenic symptoms [reflecting low blood glucose to the brain] during hypoglycaemia may show aggression because of the symptoms they are experiencing rather than because of the blood glucose level *per se*). However, no association was found in the present study between either the autonomic or the neuroglycopenic symptoms as experienced by the subjects and the change in anger-state. On the other hand, the change in autonomic and neuroglycopenic symptom scores between study conditions was highly correlated. This suggests that the factors, which determine an individual's anger-state during hypoglycaemia, are independent of those that result in symptom generation. Some support for this come from a recent study by Merbis et al. (1996) of type 1 diabetic subjects with unawareness of hypoglycaemia (i.e. loss of the classical autonomic warning symptoms of hypoglycaemia) where an overall tendency to a more negative mood-state and to increased feelings of anger were reported by subjects despite an impaired symptomatic response to hypoglycaemia. The counterregulatory hormones released during hypoglycaemia were not measured in the present study (e.g. adrenaline, noradrenaline, and glucagon) and so an analysis for correlation's between the hormonal response and anger-state was not possible. It is possible that the change in anger-state results from an effect of these hormones on the brain, although the symptom data from the present study, and the findings of Merbis et al. (1996), would not be consistent with this.

It is also possible that high insulin levels may have modified the expression of anger during hypoglycaemia. The model used to induce hypoglycaemia requires a supraphysiological dose of insulin to overcome the body's natural defence mechanisms against hypoglycaemia. By including a hyperinsulinaemic euglycaemic control condition this effect is at least partially controlled for, but this does not exclude a possible effect of insulin on mood that is specific to the hypoglycaemic state.

The subjects in this study were predominantly male which may have affected the reported findings. Normative data from the STAXI suggests that females generally report a greater baseline anger-state score than males, although this is reversed in the sub-group of subjects who were college students (Spielberger, 1991). The size of the study group in this analysis is too small to permit any examination of sex differences in response to hypoglycaemia.

In summary: -

The present study has shown a significant overall increase in anger-state during hypoglycaemia in a group of non-diabetic human subjects, whose self-reported measures of anger-trait and anger-expression fall well within the expected normal distribution. Hypoglycaemia-associated anger may adversely effect an individual's social and work relationships and may explain why some type 1 diabetic individuals refuse to be aided in the treatment of hypoglycaemia as it occurs. Future research should be aimed at examining the origins of individual differences in mood change during hypoglycaemia.

PART III

STUDY 4

Mood, appraisal and personality during acute hypoglycaemia.

The use of controlled hypoglycaemia as a biological tool for studying mood-state was first reported by Gold et al. (1995c). In their study the UWIST Mood Adjective Check List (Matthews et al. 1990) was administered to 24 non-diabetic subjects during hypoglycaemic and euglycaemic steady states using a repeated measures design. In comparison with normoglycaemia, hypoglycaemia induced a mood-state termed 'tense-tiredness', characterised by an increase in tense arousal (a measure of anxiety-calmness), a decrease in energetic arousal (a measure of subjective energy), and a decrease in hedonic tone (a measure of happiness-sadness). Other studies have confirmed in a less controlled manner the association of hypoglycaemia with negative mood states (Gonder-Frederick et al. 1989; Hepburn et al. 1995). Thayer (1989) had previously postulated that mood might consist of two principal components, tense arousal and energetic arousal, which could respond in opposite directions to produce a state of tense tiredness. The state of tense-tiredness had been implicated in the development of certain clinical states such as depression. Thayer (1989) indicated that there was a need to develop methods of inducing this mood state to validate the notion of multiple arousal systems underlying subjective reports of mood, suggesting that "tension, energy, and the associated conditions of physiological arousal might seem as variations of the same psychophysiological condition, perhaps differentiated only in terms of subjective labelling" (p.54). The

study by Gold et al. (1995c) demonstrated that acute hypoglycaemia could evoke the theoretically important mood state of tense-tiredness, and lent support to Thayer's (1989) original hypothesis. Furthermore, support was also provided for the validity of the non-orthogonal three-dimensional structure of mood proposed by Matthews et al. (1990).

The association between acute hypoglycaemia and negative mood states has now been replicated using different models of experimental hypoglycaemia (Gold et al. 1995c, Hepburn et al. 1995). It is of value now to extend this research further by examining the more cognitive aspects of the emotional changes, in particular appraisal, during hypoglycaemia. An appraisal is a subjective or objective rating of a life event, usually in terms of its degree of unpleasantness, changeability or control (Lazarus and Folkman, 1984). In this context, a life event is a 'major' event that impacts on an individual in either a positive or negative way (Holmes and Rahe, 1967). Lazarus and Folkman (1984) proposed that there are two principal types of appraisal process: primary and secondary appraisals. Primary appraisals describe how a person evaluates the nature and meaning of a particular event in relation to their well-being, whilst secondary appraisals rely on an individual's knowledge in determining how best to cope during a particular event (Lazarus and Smith, 1988). During primary appraisal a situation's potential stressfulness is assessed either in terms of emotional outcomes or as an evaluation of a situation (i.e. 'I was...' versus 'It was...'). This is called psychological and situational reasoning, respectively (Locke and Pennington, 1982). Cox and Ferguson (1991) have proposed that situational reasoning is a more theoretically sound method for the evaluation of primary appraisals. More recently, Ferguson and colleagues (Ferguson et al. 1997; Ferguson and Cox, 1993; Ferguson et al. 1998) have developed a psychometric questionnaire of primary appraisal (the Appraisal of Life Events scale [ALE]) from current theories of appraisal and previous empirical work. The authors have proposed a model of primary appraisal, which consists of three, non-orthogonal

dimensions: 'threat', 'loss' and 'challenge'. Challenge in this context refers to the degree to which the environment is perceived as allowing for personal growth and development (e.g. 'stimulating', 'enjoyable'). Threat indicates the degree to which the environment is appraised as being potentially threatening, physically harmful, and generating anxiety (e.g. 'hostile', 'worrying'). Loss refers to the potential for personal suffering. The ALE has been shown to good construct validity, internal reliability and high test-retest reliability (Ferguson et al. 1997; Ferguson and Cox, 1993; Ferguson et al. 1998). Thayer (1989) has proposed that individuals should show more negative appraisals and negative cognitions during tense tiredness. Our hypothesis was that hypoglycaemia, through its induction of the negative mood state tense-tiredness, would lead to participants' giving more negative appraisals of a life event. Thus, the study was designed to examine the effects of a controlled physiological change on the life difficulty appraisal process.

Methods

Subjects

Twenty (15 male, 5 female) healthy, non-diabetic human subjects aged 27 (21 - 42) years (median [range]) were studied. The subjects did not have any significant medical history or a family history of diabetes, nor were they taking any regular medication other than the oral contraceptive pill. Subjects were recruited by local advertisement, and were not paid for their participation in the study. The study was approved by the Healthy Volunteer Studies Medical Research Ethics Sub-Committee of the Lothian Medicine and Oncology Ethical Advisory Committee, and written consent was obtained from all of the subjects.

Procedure

All participants undertook two laboratory sessions, each at least two weeks apart, in which experimental euglycaemia was either; (i) established and maintained or (ii) hypoglycaemia then induced. Although subjects knew that they would undergo both study conditions, they were not informed which condition was being performed on each study day. A modified hyperinsulinaemic glucose clamp technique was used to achieve the experimental conditions of hypo- and euglycaemia (DeFronzo et al. 1979).

In this study arterialised whole blood glucose concentration was stabilised initially at 5.0 mmol/l for one hour for both conditions. In one condition, hypoglycaemia (2.6 mmol/l) was then induced and maintained for one hour, and in the other condition the blood glucose concentration was maintained throughout at 5.0 mmol/l (normoglycaemia). An interval of 30 minutes was interposed between baseline and each study condition to allow for the time required to achieve hypoglycaemia. Subjects were not informed of their blood glucose level at any time during the laboratory sessions. The subjects underwent the experimental conditions in a counterbalanced fashion (i.e. half the subjects underwent hypoglycaemia first followed by the normoglycaemic control condition, and half the reverse).

As subjects were recruited by local advertisement our study cohort consisted entirely of health professionals (17 doctors, 2 nurses, 1 dietician). All of our subjects were post-graduates, but none had achieved their final career post. Health professionals are in a highly competitive working environment at this stage in their careers, because of the limited number of senior posts. This may also mean that a health professional may have to move to different regions in the country in order to obtain a more senior post, which disrupts working and personal relationships. Because of the considerable stress involved in the progress of their career, we decided to ask subjects to appraise their career prospects during each of the study conditions.

Participants then completed the ALE questionnaire with respect to the challenge, threat and loss induced by this subject.

Test Battery:

(A) Patients completed four self-report questionnaires:

1. Edinburgh Hypoglycaemia Scale (Deary et al. 1993b).
2. UWIST Mood Adjective Check-list [UMACL] (Matthews et al. 1990).
3. Appraisal of Life Events Scale [ALE] (Ferguson et al. 1997; Ferguson and Cox, 1993; Ferguson et al. 1998)).
4. Eysenck Personality Questionnaire-Revised, Short Form [EPQ-R] (Eysenck et al. 1985).

(B) Psychometric tests of general cognitive function

5. Digit Symbol (DSS) (Wechsler, 1981).
6. Trail Making Test B (TM-B) (Reitan and Davison, 1974)

The EHS and measures of cognitive function (DSS and TMB tests) were used to establish that, at the predetermined level of hypoglycaemia (2.6 mmol/l), significant symptomatic and cognitive change had occurred in our study cohort, i.e. significant neuroglycopenia had been established. The UMACL was applied to compare mood-state between study conditions and in an attempt to replicate previous findings (Gold et al. 1995c). The EPQ-R was used to establish whether self-reports of the major personality dimensions remained stable between the two physiological states because of the recognised influences of personality on both appraisal and emotion (Wells and Matthews, 1994). The expectation was that mood and appraisal would alter negatively during the hypoglycaemic condition, but that subjects' self-reports of long-standing personality levels would not.

Statistical analysis

The results were analysed independently for each test of general cognitive function and for each measure of symptom score, mood, personality and appraisal. For those measures taken at baseline(s) and during study conditions (normoglycaemia and hypoglycaemia) a mixed model Analysis of covariance (ANCOVA) was used with order of session as a between-subjects factor, with two levels (normoglycaemia - hypoglycaemia or hypoglycaemia - normoglycaemia), and study condition as a within-subjects factor with two levels (normoglycaemia vs. hypoglycaemia). Test results at baseline were used as variable covariates in each analysis. For those measures taken during study conditions only, the Wilcoxon matched-pairs signed ranks test was used to compare study conditions and a Mann-Whitney used to examine for order effects. Correlation's between study conditions were performed using the Spearman's Rank Order test. An alpha level of 0.05 was used for all statistical tests.

No significant order effects were found for any of the measures and tests employed in this study.

Results

Glucose

The mean (\pm S.D) blood glucose during the hypoglycaemic clamp was 2.6 (\pm 0.2) mmol/l, and 5.0 (\pm 0.4) mmol/l during the euglycaemic clamp, showing that stable glycaemic plateau's had been achieved.

Symptoms

Significant increments in total autonomic symptom scores ($F = 34.4$, $df\ 1,18$, $p < .001$) and in total neuroglycopenic symptom scores ($F = 7.6$, $df\ 1, 18$, $p < .05$) were

recorded during the hypoglycaemia condition of the study (Table.3.4.1). No significant change occurred in symptoms of hypoglycaemia-associated malaise ($F = .25$, $df\ 1, 18$, $p = ns$). The significant increases in neuroglycopenic and autonomic symptom reports confirm that the subject group was symptomatically aware of hypoglycaemia at the predetermined blood glucose level, and more specifically were experiencing neuroglycopenia.

General cognitive function

In the present study, scores achieved on the DSS were significantly lower ($F = 31.0$, $df\ 1, 18$, $p < .001$), and times taken to complete the TM-B were significantly longer ($F = 9.8$, $df\ 1, 18$, $p < .01$) during hypoglycaemia when compared with normoglycaemia (Table.4.3.1). This confirmed, as expected, that these standard measures of general cognitive function were significantly affected at a blood glucose of 2.6 mmol/l indicating that neuroglycopenia sufficient to impair brain functioning had been established.

Personality

Table 3.4.1. shows scores recorded on each of the personality dimensions during each of the study conditions. Overall mean (S.D.) score for neuroticism during normoglycaemia was 3.4 (3.0), and during hypoglycaemia was 3.4 (3.0), which were not significantly different ($z = -0.52$, $p = 0.60$). Overall mean (S.D.) score for extraversion during normoglycaemia was 7.7 (3.5), and during hypoglycaemia was 7.7 (3.5); ($z = -0.09$, $p = 0.92$). Mean (S.D.) score for psychoticism during normoglycaemia was 2.6 (1.6), and during hypoglycaemia was 3.0 (1.9); ($z = -1.4$, $p = 0.16$). These results indicate that subjects' reports of personality dimensions remain stable between the two physiological states. A Spearman's Rank Order Correlation was performed to assess whether this stability in personality dimensions also held true for individual subjects (i.e. whether stability of group means obscured considerable variability in individual responses to the hypoglycaemia condition).

Spearman's r_s for extraversion, neuroticism and psychoticism personality traits were 0.76 ($p < 0.001$), 0.58 ($p < 0.05$), and 0.74 ($p < 0.001$) respectively. These results indicate that individual personality dimensions also remained reasonably constant between study conditions.

Mood

The hypoglycaemic condition evoked a negative mood state and produced a clear separation of the three dimensions of mood (Table.3.4.1). Measures of hedonic tone significantly decreased during the hypoglycaemic condition ($F = 21.2$, $df\ 1,18$, $p < 0.01$), whereas measures of tense arousal significantly increased ($F = 41.2$, $df\ 1,18$, $p < 0.01$). Energetic arousal did not alter significantly during hypoglycaemia ($F = 1.4$, $df\ 1,18$, $p = 0.25$).

This result confirms the previous finding of the separation of each of the three mood dimensions during hypoglycaemia, although the lack of a significant change in energetic arousal was unexpected (Gold et al. 1995c). In this experiment hypoglycaemia has induced a negative mood state characterised by increased tension and decreased happiness, but has had no significant effect on subjective energy.

Appraisal

The results of the subjects' appraisal of their career under the two study conditions are shown in Table 3.4.1. The mean (S.D.) score on the challenge sub-scale during normoglycaemia was 20.2 (5.2), and during hypoglycaemia was 17.8 (6.0), which were significantly different ($z = -2.05$, $p = 0.04$). Mean (S.D.) score on the threat sub-scale during normoglycaemia was 9.3 (5.3), and during hypoglycaemia was 12.1 (4.9); ($z = -2.73$, $p = 0.006$). Mean (S.D.) score on the loss sub-scale during normoglycaemia was 3.0 (2.2), and during hypoglycaemia was 5.1 (3.7); ($z = -2.76$, $p = 0.006$). Spearman's rank order correlation was performed on individual scores on each subset of the ALE between study conditions. Spearman's r_s for Challenge,

Threat and Loss were 0.53 ($p < 0.05$), 0.74 ($p < 0.001$), and 0.68 ($p < 0.005$) respectively.

These analyses indicate that, for this group of subjects, the physiological state of hypoglycaemia, in comparison with normoglycaemia, has evoked significantly more negative appraisals of a life problem. They also show that this group effect does not obscure significant individual variation in response to the study condition.

Table 3.4.1

Measures of general cognitive performance, hypoglycaemia symptoms, personality, mood and appraisal. Values are shown as mean (standard deviation).

	EUGLYCAEMIC STUDY		HYPOGLYCAEMIC STUDY		
	Baseline	Euglycaemia	Baseline	Hypoglycaemia	
DSS (score)	69.9(14.0)	73.2(14.6)	68.9(14.8)	66.1(12.5)	p<.05
TM-B (secs)	30.5(13.4)	28.2(14.4)	31.35(15.7)	35.6(16.2)	p<.05
Autonomic	8.5(2.6)	7.8(2.0)	8.4(3.6)	14.2(6.5)	p<.05
Neuroglycopenic	8.2(3.4)	8.0(5.0)	8.1(4.7)	10.6(5.7)	p<.05
Neuroticism		3.4(3.0)		3.4(3.0)	p=ns
Extraversion		7.7(3.5)		7.7(3.5)	p=ns
Psychoticism		2.6(1.6)		3.0(1.9)	p=ns
Tense Arousal	13.7(4.5)	13.6(4.7)	15.5(5.2)	22.6(4.4)	p<.05
Hedonic Tone	25.8(3.6)	26.8(2.7)	26.2(4.4)	22.8(4.3)	p<.05
Energetic Arousal	17.4(5.2)	16.8(4.6)	16.8(5.1)	18.2(4.4)	p=ns
Challenge		20.2(5.2)		17.8(6.0)	p<.05
Threat		9.3(5.3)		12.1(4.9)	p<.05
Loss		3.0(2.1)		5.1(3.7)	p<.05

Discussion

The present study has demonstrated that, during controlled hypoglycaemia, a specific life problem may be viewed significantly more negatively. The professional careers of this group of health care workers were subjectively appraised as less challenging, as potentially more likely to generate anxiety and threat, and more likely to entail personal loss while the subjects were in a state of hypoglycaemia. The present study also lends support to the hypothesis that hypoglycaemia, through its evocation of a negative mood state, would lead to more negative appraisals of a life event. Furthermore, it has been demonstrated that personality trait levels are preserved during experimental hypoglycaemia, and are not altered by the induction of a negative mood and appraisal state.

The direction of change observed in each of the ALE sub-scales during hypoglycaemia provides useful information to the debate concerning the nature of the relationship between mood and emotion. Some authors have suggested that feelings of stress should be positively correlated to appraisal of threat, loss and challenge (Lazarus and Folkman, 1984), whereas others have suggested that feelings of stress should only be related to threat and loss appraisals (Dienstbier, 1989). Ferguson et al. (1997) have examined the relationship between mood and situational appraisal in a study of 71 blood donors. The Stress-Arousal Checklist (SACL) (Cox and Mackay, 1985) and the ALE were applied to the subjects who were asked about their perceptions and feelings about blood donation. The SACL assesses mood along two orthogonal dimensions: stress and arousal. A high score on the stress dimension indicates feelings of unpleasantness and high scores on the arousal scale indicate feelings of being alert and awake. Ferguson, Matthews, and Cox (Ferguson et al. 1998) found that in blood donors stress was positively correlated with negative appraisals (threat and loss) and not with challenge. Challenge was positively

associated with arousal. The findings of the present study, and those of Ferguson, Matthews, and Cox (1998), would tend to support the hypothesis of Dienstber (1989) and suggest that stress can have both positive and negative aspects.

In the present study hypoglycaemia has produced a negative mood state characterised by increased tension and decreased happiness. Unlike previous work (Gold et al. 1995c) no change in the subjects' energetic arousal was seen. This finding is surprising because one would intuitively expect neuroglycopenia, which is ordinarily associated with drowsiness and low cortical arousal, to cause a decrease in energetic arousal, and indeed this was the finding in previous work using the same model (Gold et al. 1995c). It is recognised that there is considerable inter-individual variability in cognitive (Gonder-Frederick et al. 1994) and mood (Gonder-Frederick et al. 1989) changes during hypoglycaemia. Moreover, the thresholds (blood glucose level) at which autonomic and neuroglycopenic symptoms develop, and cognitive function deteriorates, during experimental hypoglycaemia also show inter-individual variability (Gonder-Frederick et al. 1994). In this regard, it is interesting to note that the group examined by Gold et al. (1995c) showed a much greater increase in neuroglycopenic scores than the present study group during the same degree of hypoglycaemia. The participants in this study completed the UMACL towards the end of the 60 minute clamp period which would compare with the third time points in the two hypoglycaemic conditions used in the study by Gold et al. (1995c). At this time point Gold et al (1995c) reported a *doubling* in reported neuroglycopenia symptoms from baseline ($p < 0.001$), whereas an increase in neuroglycopenic symptoms of $\cong 25\%$ only ($p < 0.05$) was noted in the present study. Furthermore, because mood and symptoms were assessed at multiple time points they were able to show that energetic arousal *fell* progressively through the 60 minutes of the hypoglycaemic clamp and that this was matched by a continuing *rise* in neuroglycopenic symptoms. The authors suggested that the different time courses shown by the three dimensions of mood might indicate different biological factors

were involved in their genesis. The present study could therefore be seen as providing further evidence of the centrality of cortical arousal to the generation of the mood state of energetic arousal, and of the existence of different arousal systems. It is also clear that, during controlled hypoglycaemia, both the degree and duration of hypoglycaemia affect mood-states and this should be taken into consideration in future research in this area.

The present study has provided further evidence of the usefulness of controlled hypoglycaemia as a model for manipulating stress and emotion in human subjects. Hypoglycaemia has been shown to produce negative mood states and associated negative appraisals and this might allow for future study into the biological bases of mood, and the effect of pharmacological interventions. Despite the absence of a change in energetic arousal the present study still found more negative appraisals during hypoglycaemia which suggests that changes in tense arousal and hedonic tone might be more important influences on primary appraisal.

In summary: -

These findings add to the expanding literature on the non-cognitive psychological effects of acute hypoglycaemia. It is now quite clear that acute hypoglycaemia can evoke negative stresses and emotions in human subjects which may have an adverse influence on an individual's social and work interactions. In particular, the present findings show that, in non-diabetic subjects, acute hypoglycaemia induces a negative mood state that leads to more negative appraisals of life events. Type 1 diabetic subjects may not show such negative appraisals during hypoglycaemia because of prior experience of the hypoglycaemic state or other factors related specifically to the diabetes per se. However, type 1 diabetic subjects also show a tendency towards negative mood states during acute hypoglycaemia (Gonder-Frederick et al. 1989) and as such it appears likely that they might report similar negative appraisals. Future studies should be directed at examining the interaction between negative

stresses and emotions during acute hypoglycaemia in type 1 diabetic subjects and should also examine for the potential psychomorbidity of cumulative episodes of recurrent acute hypoglycaemia.

PART III

STUDY 5

A comparison of the symptoms of hypoglycaemia induced by the one-step hyperinsulinaemic glucose clamp and the insulin-infusion techniques in type 1 diabetic and non-diabetic subjects.

It has been recognised since the introduction of insulin in the 1920s that ‘a characteristic train of symptoms’ occurs when the blood glucose falls to beneath the normal range. These symptoms were so typical of a low blood sugar that they were termed the ‘hypoglycaemic reaction’ (Fletcher and Campbell, 1922). It is now recognised that distinct symptom sub-types represent the ‘hypoglycaemia reaction’. However, some controversy has arisen as to which symptoms most characterise an individuals symptomatic response to hypoglycaemia.

Two retrospective analyses of large numbers of type 1 diabetic subjects lead to the development of the Edinburgh Hypoglycaemia Scale (Hepburn et al. 1992; Deary et al. 1993b). This has provided one means by which studies between centres can be compared. However, differences do exist between the Edinburgh Hypoglycaemia Scale, derived from the retrospective reports of individuals with type 1 diabetes, and symptoms derived from experimental studies. Towler et al. (1993), using the one-step hyperinsulinaemic glucose clamp to examine the symptomatic response to hypoglycaemia, suggested that it could best be divided into two sub-groups, viz.

autonomic [or neurogenic] and neuroglycopenic symptoms, both of which have distinct physiological bases. This compares with the Edinburgh Hypoglycaemia Scale, which suggested three symptom subgroups; autonomic, neuroglycopenic and hypoglycaemia-associated malaise symptoms (Deary et al. 1993b). Using the insulin-infusion technique to induce hypoglycaemia in 30 type 1 diabetic and 25 non-diabetic subjects Hepburn et al. (1991a) also suggested that the hypoglycaemic reaction could be represented by two distinct symptom sub-types; autonomic and neuroglycopenic. However, this analysis differed from that of Towler et al. (1993) in that Hepburn et al. (1991a) found the symptom warmth to be autonomic whereas Towler et al. (1993) found it to be neuroglycopenic. Hepburn et al. (1991a) also found the symptom weakness did not group clearly into either symptom sub-type whereas Towler et al. (1993) suggested it was clearly a neuroglycopenic symptom. Furthermore, Hepburn et al. (1991a), suggested that palpitation (pounding heart) was not a good symptom of hypoglycaemia and they did not include the symptom tingling. The differences between these two studies may have arisen because of the different techniques employed, and also because of the nature of the subjects studied (non-diabetic vs. a mixed group of type 1 diabetic and non-diabetic subjects).

In order to clarify this area, the symptom reports from a large number of type 1 diabetic and non-diabetic subjects who have participated in hypoglycaemia research in our centre over the last few years have been analysed. By combining these studies, the power of our analysis to discover differences in the symptomatic response to hypoglycaemia, if they are apparent, between the two methods of inducing hypoglycaemia, and also between type 1 diabetic and non-diabetic subjects is significantly increased.

Methods

The analysis in this study is based on the hypoglycaemia symptom reports of 154

(78 type 1 diabetic; 76 non-diabetic) subjects who had participated in studies in our centre over the last few years. None of the type 1 diabetic subjects included in this analysis had reported a history of hypoglycaemia unawareness, or a change in symptom pattern and intensity since commencing insulin therapy. All type 1 diabetic subjects were using human insulin for their insulin replacement regimen, and type 1 diabetic subjects were excluded if they were taking any medication that may have interfered with their symptomatic response to hypoglycaemia (e.g. beta-adrenoreceptor blockers), or if they had any clinical evidence of microvascular disease. None of the type 1 diabetic subjects had clinical evidence of autonomic neuropathy, as assessed by standard cardiovascular reflex tests (Ewing et al, 1982). In those studies designed to compare different types of insulin only those subjects given human insulin have been included. The non-diabetic subjects were all healthy and on no regular medication other than the oral contraceptive pill. None had a family history of diabetes, or a history of neurological or cardiovascular disease. The age of our subject group ranged from 20-55 years.

The methods used to induce hypoglycaemia in two of the studies from which hypoglycaemia symptom reports were taken for the present analysis have been described in detail elsewhere (MacLeod et al. 1994, Gold et al. 1995). Hypoglycaemia symptom reports were also taken from subjects participating in those studies reported in this thesis (visual and auditory information processing studies; pp 101-140) and from studies currently being conducted in the Department of Diabetes, Royal Infirmary of Edinburgh (Ewing *et al.* unpublished data). The hyperinsulinaemic glucose clamp involves the continuous infusion of insulin at a fixed rate (following a priming ramped infusion to saturate insulin receptors) and a glucose infusion at a variable rate which is appropriately adjusted to maintain the blood glucose at a desired level. In all of the studies included in this analysis subjects have been maintained at euglycaemia (5.0 mmol/l) for a period of 45-60 minutes prior to being dropped in a single step to hypoglycaemia (2.6 mmol/l) where

they have been maintained for a further 45-60 minutes. With the insulin infusion technique a continuous, unopposed infusion of insulin is delivered until there is evidence of autonomic activation. At this point a dextrose infusion is often started to aid in the restoration of euglycaemia. The time of autonomic activation is identified by the rapid onset of sweating (measured by a hygrometer) and increase in heart rate (measured by precordial leads) (Hepburn, 1993). In all of the studies included in this analysis it is arterialised blood glucose recordings that are being reported, and all have used human insulin to induce hypoglycaemia.

The hypoglycaemic questionnaire used in these studies was developed in Edinburgh over the last few years (Hepburn et al. 1991a; Hepburn et al. 1992; Deary et al. 1993b). During hypoglycaemia subjects are asked to complete the questionnaire, and to rate the presence or absence of the symptoms they are experiencing along a 7-point Likert-like scale (where 1 = symptom not experienced at all, and 7 = symptom experienced with maximum intensity). This questionnaire is given to subjects after they have been stabilised for 15 minutes at a predetermined level of hypoglycaemia in the hyperinsulinaemic glucose clamp studies, and when there is evidence of autonomic activation in the continuous insulin infusion studies. Because this questionnaire evolved over repeated studies the symptoms used are not identical in each study. The symptom headache was only used in later studies. In the 69 subjects who reported on this symptom during hypoglycaemia only 15 (22%) responded positively, with a mean intensity of only 0.3. Inco-ordination and odd behaviour were also only reported by a small number of subjects, and hence all three of these symptoms were rejected from further analysis. The symptoms used in this analysis were; hunger, inability to concentrate, weakness, warmth, drowsiness, trembling, sweating, tired, confusion, blurred vision, palpitation, anxiety, nausea, tingling lips, difficulty speaking and double vision.

Statistical Analysis

Statistical analysis was performed on the Statistical Package for the Social Sciences (SPSS/PC + 2.0), SPSS (Chicago, Illinois, U.S.A). Symptom frequency and intensity were compared using Spearman's rank correlation for non-parametric data. Principal components analysis (PCA), followed by an orthogonal varimax rotation was used in an attempt to ascertain whether any latent structure could be identified in the hypoglycaemia symptom-profiles of type 1 diabetic and non-diabetic subjects, and during the insulin-infusion or hyperinsulinaemic glucose clamp studies. Finally, PCA was applied to the group as a whole. PCA begins with a correlation matrix where scores for every symptom are correlated with those for every other symptom. Sub-groups of symptoms that intercorrelate relatively highly are revealed, suggesting certain grouping with a common underlying cause. For each of the following separate analyses the first unrotated principal component revealed that all of the symptoms loaded moderately or highly confirming that they were all good indicators of hypoglycaemia. Factor analysis is then used to determine if, within the main unrotated principal component, there is evidence of any sub-grouping of variables (e.g. autonomic or neuroglycopenic symptoms). Each factor is given an eigen value and these can be plotted graphically (Scree slope). In theory, it is possible to produce an infinite number of factors from an analysis such as this. Traditionally, factors with eigen values greater than 1 have been taken to be significant, however, from the scree slope one is usually able to determine how many factors best represent the latent structure of the variables in the analysis. Where the slope of the curve flattens is usually the point at which each additional factor adds little to the overall variance in the analysis.

Results

In the following section results are presented separately for the two subject groups (type 1 diabetic vs. non-diabetic subjects) and for the two experimental techniques (hyperinsulinaemic glucose clamp studies vs. insulin infusion studies). A final analysis of the pooled data from all subjects is then presented.

Symptom frequency and mean intensity

1. Type 1 diabetic patients (N=78) vs. non-diabetic subjects (N=76)

The symptoms reported with the greatest frequency by type 1 diabetic patients were hunger (91%), inability to concentrate (81%), warmness (76%) and drowsiness (73%), and those reported with the least frequency were nausea (25%), tingling lips (19%), and difficulty speaking (15%). The non-diabetic subjects reported hunger (92%), inability to concentrate (80%), tiredness (75%), and trembling (74%) with the greatest frequency, and double vision (8%), tingling lips (11%), and difficulty speaking (19%) the least (Table 3.5.1). The symptoms reported with the highest mean intensity (maximum score 7; minimum score 1) by the type 1 diabetic subjects were hunger (4), inability to concentrate (3), weakness (3) and warmness (3), and those reported with the highest mean intensity by the non-diabetic subjects were hunger (4), trembling (3), warmness (3) and sweating (3) (Table 3.5.1).

There was very close agreement between type 1 diabetic and non-diabetic subjects when frequency of reported symptoms were correlated ($\rho = 0.74$; $p < 0.01$).

Similarly type 1 diabetic and non-diabetic subjects showed close agreement when mean ($\rho = 0.61$; $p < 0.05$) and standard deviation ($\rho = .45$; $p = 0.14$) of the intensity of reported symptoms were compared. These results indicate that during hypoglycaemia both type 1 diabetic and non-diabetic subjects are experiencing a

similar symptomatic response in terms of the type of symptoms they report, the intensity with which they report those symptoms and the variability of their responses.

2. Hyperinsulinaemic glucose clamp studies (N = 88) vs. insulin infusion studies (N = 66).

The symptoms reported with the greatest frequency during the hyperinsulinaemic glucose clamp studies were hunger (83%), inability to concentrate (77%), drowsiness (70%) and trembling (67%). Those reported with the least frequency were tingling lips (20%), double vision (22%) and nausea (25%) (Table 3.5.1). Similarly the symptoms reported with the greatest frequency during the insulin infusion studies were hunger (89%), inability to concentrate (85%), warmth (77%) and trembling (71%). Those reported with the least frequency were tingling lips (12%), nausea (24%) and double vision (26%) (Table 3.5.1).

There was also close agreement between subjects during both techniques in terms of the frequency ($\rho = 0.69$; $p < 0.05$), mean intensity ($\rho = 0.83$; $p < 0.01$) and variability ($\rho = 0.63$; $p < 0.05$) of the symptom responses.

Factor analysis

Principal components analysis (PCA) was applied to the symptom scores of each separate sub-group (type 1 diabetic, non-diabetic, clamp and infusion groups) and to the study population as a whole in order to examine the latent structure of the hypoglycaemic response. Only those symptoms which were reported by more than 25% of the study group were included in this analysis, as these were felt to be better discriminators of the hypoglycaemic response (the symptoms 'tingling lips', 'nausea', 'difficulty speaking', and 'double vision' were rejected at this stage). For

each analysis in this study, the resulting scree slopes are shown in Figure 3.5.1. It is very clear from this that each analysis reveals one very significant factor that accounts for between 43-48% of the shared variance. The significance of this factor is such that any attempt to reduce the variables to a structure containing more than one factor would not be reliable (despite some of the other factors having eigen values greater than 1).

1. Principal components analysis of the symptoms reported by the type 1 diabetic subjects during hypoglycaemia revealed only one factor which had an eigen value of 6.2 and accounted for 48.0 % of the variance in responses (Table 3.5.2). Similarly, PCA of the non-diabetic subjects revealed only one factor with an eigen value of 5.6 accounting for 43.0 % of the variance (Table 3.5.2). PCA of the symptom reports resulting from the hyperinsulinaemic glucose clamp experiments also revealed one factor with an eigen value 6.0, and accounting for 46.0 % of the variance. PCA of the symptom reports during the insulin-infusion technique also suggested that a single factor would best account for the latent structure of the responses. This factor accounted for 43.0 % (eigen value 5.6) of the shared variance (Table 3.5.2).

Finally, PCA was applied to all of the symptom reports (N=154). Once again all of the symptoms reported loaded moderately or highly on the first unrotated principal component confirming their usefulness in assessing hypoglycaemia (Table 3.5.2). This analysis revealed that one factor alone best described the structure of the overall symptom response, i.e. the subjects recognised that they were hypoglycaemic, but did not differentiate between autonomic or neuroglycopenic symptoms.

Table 3.5.1 Frequency and mean intensity of hypoglycaemia symptoms reported during experimental hypoglycaemia (note; mean intensity scores have been adjusted so that the minimum score is 0, the maximum 6). Only those symptoms, which were then used in factor analysis, are included. The 5 most intensely experienced symptoms for each group are highlighted in bold. (F = Frequency; I = Mean Intensity).

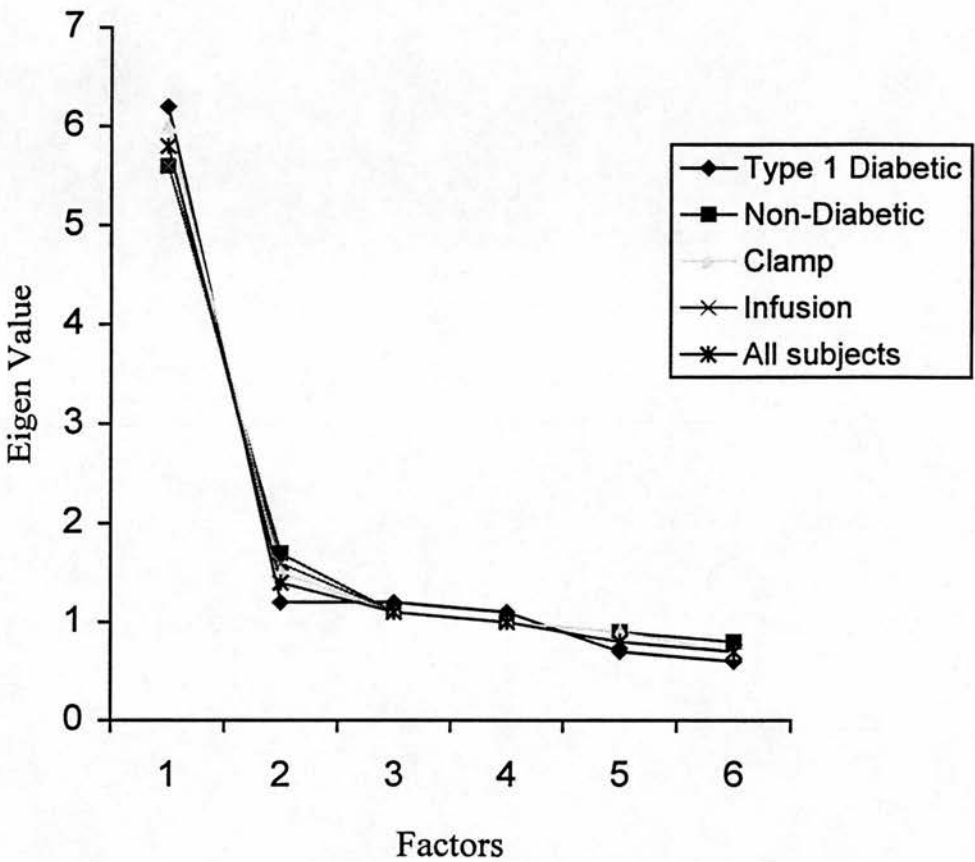
SYMPTOM	TYPE 1 DIABETIC		NON- DIABETIC		CLAMP		INFUSION	
	<i>F</i>	<i>I</i>	<i>F</i>	<i>I</i>	<i>F</i>	<i>I</i>	<i>F</i>	<i>I</i>
Hunger	91	3.0	92	3.3	83	3.2	89	3.0
Inability to concentrate	81	1.8	80	1.9	77	1.8	85	2.0
Weakness	72	1.8	63	1.4	67	1.6	68	1.7
Warmness	76	1.8	67	2.0	67	1.8	77	1.8
Drowsiness	73	1.7	64	1.5	70	1.6	67	1.6
Trembling	64	1.6	74	2.0	67	1.8	71	1.9
Sweating	63	1.6	68	2.0	65	1.8	67	1.9
Tired	72	1.6	75	1.8	81	1.8	64	1.6
Confusion	60	1.3	49	1.0	47	1.0	65	1.4
Blurred vision	55	1.2	45	0.8	44	0.8	58	1.2
Palpitation	56	1.2	64	1.6	60	1.4	62	1.3
Anxiety	46	0.9	57	1.4	52	1.1	50	1.0

Table 3.5.2. Results of factor analysis applied to symptom reports of subjects during experimental hypoglycaemia.

	TYPE 1 DIABETIC	NON- DIABETIC	CLAMP	INFUSION	ALL SUBJECTS
<i>Symptom</i>	<i>Factor</i>	<i>Factor</i>	<i>Factor</i>	<i>Factor</i>	<i>Factor</i>
Anxiety	0.66	0.63	0.68	0.62	0.64
Blurred Vision	0.75	0.59	0.67	0.68	0.68
Confusion	0.70	0.74	0.71	0.69	0.71
Difficulty Speaking	0.67	0.75	0.73	0.69	0.70
Drowsiness	0.76	0.68	0.65	0.76	0.70
Hunger	0.57	0.42	0.48	0.50	0.50
Inability to concentrate	0.75	0.75	0.79	0.67	0.74
Pounding heart	0.76	0.53	0.62	0.69	0.64
Sweating	0.47	0.60	0.61	0.40	0.53
Tiredness	0.62	0.70	0.66	0.64	0.65
Trembling	0.72	0.64	0.69	0.68	0.68
Warmness	0.64	0.56	0.60	0.54	0.59
Weakness	0.83	0.79	0.80	0.81	0.81
Eigen value	6.2	5.6	6.0	5.6	5.8
Percentage of total variance	47.8	43.0	46.0	43.0	44.4

Figure 3.5.1

This Scree Slope shows the eigen values plotted against factor number for each rotation in the factor analysis for each of the sub-groups and for the group as a whole.



Discussion

Research into the symptomatic response to hypoglycaemia has essentially taken two forms; retrospective analysis of symptoms reported by ambulant type 1 diabetic subjects in the community and analysis of symptoms reported by subjects (type 1 diabetic and non-diabetic) during experimental hypoglycaemia. The Edinburgh Hypoglycaemia Scale (Deary et al. 1993b), although originally derived from experimental studies (Hepburn et al. 1991a), is mainly the product of the analysis of large numbers of type 1 diabetic subjects in the community. This symptom questionnaire has proven a robust measure of the symptomatic response to hypoglycaemia as experienced by type 1 diabetic subjects in their day-to-day activities (Deary et al. 1993b).

There is considerable agreement between the findings from analysis of symptom reports and from experimental studies. For instance, physiological tremor (shaking) is recognised to increase during hypoglycaemia (Heller et al. 1987) and is similarly increased by an infusion of adrenaline (Fellows et al. 1986). However, differences do exist between the symptoms reported during experimentally induced hypoglycaemia and hypoglycaemia experienced in the community. In many ways this is not surprising given the nature of hypoglycaemia in the two different environments (one taking place in a controlled laboratory environment, the other in every day life where a multitude of other influences can effect the symptomatic response). Towler et al. (1993), for instance, found that symptoms of hypoglycaemia associated malaise (nausea and headache) did not change significantly during hypoglycaemia. Similarly, Hepburn et al. (1991a) suggested headache was a neuroglycopenic symptom and nausea an autonomic symptom. Neither symptom was reported with great frequency or intensity (Hepburn et al. 1991a). In this analysis both headache and nausea were excluded because of the infrequency with

which they were reported, and their marginal increase in intensity when they were experienced. This indicates that headache and nausea are not reliable discriminators of experimental hypoglycaemia, and that neither type 1 diabetic, nor non-diabetic, individuals are experiencing a specific hypoglycaemia-associated malaise during experimental hypoglycaemia.

Earlier analysis of the symptomatic response to experimental hypoglycaemia had also suggested that there may be differences between experimental techniques and between type 1 diabetic and non-diabetic subjects (Towler et al. 1993; Hepburn et al. 1991a). The results from this analysis indicate that the symptomatic response to hypoglycaemia induced by either technique, and between type 1 diabetic subjects with intact hypoglycaemia awareness and non-diabetic subjects, is very similar. Furthermore, during hypoglycaemia induced by either the continuous insulin infusion or the hyperinsulinaemic glucose clamp technique, type 1 diabetic subjects with intact hypoglycaemia awareness and non-diabetic subjects are unable to differentiate between autonomic and neuroglycopenic symptoms.

In summary:-

Based on the data pooled from a large number of studies involving experimental hypoglycaemia, the findings of this analysis would suggest the following symptoms as being most discriminatory of the symptomatic response to hypoglycaemia; hunger, inability to concentrate, weakness, warmth, drowsiness, trembling, sweating, tired, confusion, blurred vision, palpitation and anxiety. Furthermore, type 1 diabetic and non-diabetic subjects appear to be unable to distinguish between classical autonomic or neuroglycopenic symptoms if either a continuous insulin infusion or a one-step hyperinsulinaemic glucose clamp induces hypoglycaemia

PART IV

CLINICAL STUDY

PART IV

STUDY 6

Symptoms of hypoglycaemia in children with type 1 diabetes **mellitus**

Hypoglycaemia engenders fear and apprehension in most diabetic patients treated with insulin (Pramming et al. 1991), and is of particular concern to the parents of children with type 1 diabetes. Hypoglycaemia in children may be difficult to identify as the manifestations may be represented principally by behavioural change, such as being irritable or naughty. A young child may be unable to describe physiological changes which adults recognise as typical symptoms of hypoglycaemia, and symptoms change with age.

Symptoms of hypoglycaemia in adults are idiosyncratic, and vary in intensity and nature among individuals (Hepburn, 1993; Pennebaker et al. 1981; Hepburn et al. 1992). However, within the insulin-treated diabetic population, acute hypoglycaemia presents as a characteristic symptom complex. The development of a reliable and valid system for measurement of symptoms, essential for comparative studies of hypoglycaemia, has proved to be difficult. The application of statistical techniques such as Principal Components (Factor) Analysis to examine symptoms of hypoglycaemia in large groups of type 1 diabetic adults has permitted an objective allocation of symptoms to specific subgroups (Hepburn et al. 1992; Hepburn et al. 1991a; Deary et al. 1993b). The *Edinburgh Hypoglycaemia Scale* has been devised from these analyses and provides a possible means by which standardisation may be achieved across research centres (Deary et al. 1993b). Hypoglycaemia symptoms in

this scale are subdivided into those generated by activation of the autonomic nervous system ('autonomic' or 'neurogenic' symptoms), those that occur through the direct effect of glucose deprivation on the brain ('neuroglycopenic' symptoms), and those that are less clearly defined and represent hypoglycaemia-associated *malaise*. (see Part I, pp.10-13, for detailed discussion). It is not known whether the symptoms of hypoglycaemia in children with type 1 diabetes can be classified this way.

In addition to the scientific value of an objective examination of the symptoms of hypoglycaemia in type 1 diabetic children, the practical importance of conveying accurate information about the symptoms and possible manifestations of hypoglycaemia to the parents of the children is paramount, as most children are heavily reliant on parental assistance for treatment. This study examined the latent structure of the symptoms of hypoglycaemia experienced by type 1 diabetic children and compared this with the signs observed by their parents.

Methods

One hundred consecutive children, all of whom were accompanied by at least one of their parents, attending a paediatric diabetic out-patient clinic at the Royal Hospital for Sick Children, Edinburgh, were recruited for this study. All 100 children and 100 parents gave informed consent to participate in the survey. All of the children had type 1 diabetes and had experienced symptomatic hypoglycaemia. The children were of median (range) age 10.3 (1.5-16) years, with duration of diabetes 4.2 (0.3 - 13.0) years. At the time of questioning, mean (standard deviation) total glycated haemoglobin for the study group was 10.3 (0.3) %.

The children and parents were questioned separately. Only one parent of each child was interviewed. Each parent was questioned about the symptoms and signs of hypoglycaemia, which they considered their child to have experienced on several

occasions. Many of the diabetic children were too young, or not available, to respond appropriately to the symptom questionnaire but 43 (43%) children were sufficiently mature to document the symptom profile. This judgement on individual capability was made by the interviewer at the time of examination and was based upon a subjective assessment of the child's intellectual capabilities and maturity, however it is possible that the responses of some of the younger children may have been less reliable.

As expected the subgroup of children who were able to respond to the symptom questionnaire were on average older than the non-responding group. No significant differences were observed in duration of diabetes, total insulin dose or mean total glycated haemoglobin concentration between either group (Table 4.1.1). Total glycated haemoglobin was measured using high-speed liquid chromatography based on an ion-exchange reverse-phase partition method (Hi Auto A1c 8121) (The non-diabetic range for our laboratory was 4.5-8.0 %).

Structured questionnaire

The type 1 diabetic children and their parents were interviewed using a structured questionnaire. The parent was asked to provide sociodemographic details of the child's history of diabetes and management including total daily insulin dose, number of injections and method of glycaemic monitoring. Parents were also asked to rate their own ability to detect the onset of hypoglycaemia as it occurred in their child using a 7-point linear scale (1 = always able to detect onset of hypoglycaemia, 7 = never able to detect onset of hypoglycaemia), and to compare this with their ability to detect hypoglycaemia when their child had commenced insulin therapy after diagnosis. The frequency and morbidity (e.g. broken bones, head injury etc) of previous episodes of severe hypoglycaemia were documented. Severe hypoglycaemia was defined by the requirement to treat with glucagon or glucose by

injection, loss of consciousness, convulsion or transient hemiparesis. This differs from the current definition used in adult diabetic patients in which severe hypoglycaemia is determined by the need for external assistance to treat the patient. In the present study it was considered inappropriate to apply the definition of severe hypoglycaemia for adults to diabetic children because most young children require assistance to treat hypoglycaemia of any degree of severity.

The parents, and where possible the children, were shown a list of 23 possible symptoms of hypoglycaemia derived from published reports (Hepburn, 1993; Pennebaker et al. 1981; Hepburn et al. 1992; Hepburn et al. 1991a; Deary et al. 1993b; Macfarlane et al. 1989; Aman et al. 1989) and were asked to indicate which symptoms the child experienced regularly, and to indicate the intensity of each symptom experienced on a 7-point scale. Two 'dummy' symptoms (itching and having hiccups), unrelated to hypoglycaemia, were included in the questionnaire to check the appropriateness of individual responses. The parents were asked in addition to score two physical signs, pallor and bed-wetting, which were not shown to the children. Symptoms which a child had difficulty in comprehending were explained by the interviewer (e.g. 'nausea' was described as 'feeling sick'). Following completion of the questionnaire, the children and their parents were asked to describe any other unlisted symptoms of hypoglycaemia relevant to them. In an attempt to ascertain the typical symptom complex of individual children, both the parent and the child were asked to describe the 'usual' symptoms of hypoglycaemia with which they were familiar.

Statistical Analysis

Statistical analysis was performed on the Statistical Package for the Social Sciences (SPSS/PC + 2.0), SPSS (Chicago, Illinois, U.S.A). Results are expressed as mean \pm standard deviation or median (90% ICR) as appropriate. The comparison of factors

associated with hypoglycaemia was performed either by parametric statistical methods (ANOVA), or non-parametric statistical methods (Chi - squared and Kruskal - Wallis), as appropriate. Symptom frequency and intensity were compared using Spearman's rank correlation for non-parametric data. Principal components analysis, followed by an orthogonal varimax rotation was used in an attempt to ascertain whether any latent structure could be identified in the responses of the children and their parents to their hypoglycaemia symptom-profiles. The number of factors was determined using the scree test, and a symptom loading of ± 0.3 on any factor was considered to be significant.

Results

The parents reported that forty-five (45%) children had experienced at least one episode of severe hypoglycaemia, and 11 (11%) children had experienced five or more episodes of severe hypoglycaemia at any time since commencing treatment with insulin. Of those children exposed to severe hypoglycaemia, approximately half had experienced nocturnal episodes with 8 (18%) patients having experienced 5 or more nocturnal hypoglycaemic events. Of those suffering a severe hypoglycaemic event 22 (49%) had been treated in hospital and 5 (11%) had required multiple admissions to hospital. Six (6%) children had suffered some form of hypoglycaemia-related injury, most of which were minor head injuries. Twenty-seven (27%) children had experienced problems at school, which were attributed to hypoglycaemia (e.g. loss of schooling, poor educational performance or difficulty in concentrating during classes).

Many of the hypoglycaemic events were related to simple errors in 'self-management' of diabetes. A total of 98 (98%) of the parents were able to identify a specific cause (e.g. delayed or missed food) which they had considered to be responsible for many episodes of hypoglycaemia, but a retrospective analysis of

causation was not undertaken because of dubiety about the reliability of accuracy of recall. However, seven (7%) of the parents described their child as having deliberately induced hypoglycaemia through manipulative behaviour (e.g. refusing food) and two diabetic children (2%) were reported to have done this repeatedly.

Factors associated with severe hypoglycaemia

The frequency of severe hypoglycaemia was associated with age, with a trend towards an increase in frequency with advancing age, although this did not reach statistical significance ($p=0.06$). A higher frequency of severe hypoglycaemia was reported in those children who had developed type 1 diabetes at an earlier age ($p=0.05$), and in those children whose parents reported themselves as being less able, compared with other parents, to perceive the onset of hypoglycaemia in their child ($p=0.05$). The ability of the parental group to perceive the onset of hypoglycaemia improved with duration of the child's disease ($p < 0.001$). No correlations were observed between mean total glycated haemoglobin or insulin dose at the time of the survey, and the frequency of severe hypoglycaemia .

Symptoms and signs of hypoglycaemia: Correlation of symptom-profiles of children and those observed by their parents

The frequencies and mean intensities of the symptoms reported by the children and signs observed by the parents are shown in Table 4.1.2. Pallor during hypoglycaemia was observed by the parents with greatest mean intensity and frequency (88% of children were affected). The signs that were observed by the parents with the greatest intensity were irritability, sweating, tearfulness, hunger and, being argumentative or aggressive. The most frequent signs reported by the parents were sweating, tearfulness, irritability, hunger, and poor concentration. By comparison the children reported weakness, hunger, sweating, trembling, and

dizziness as having the greatest intensity, while their most frequent symptoms were weakness, trembling, dizziness, sleepiness and poor concentration. When the symptom frequency ranks for parents and children's estimations were compared using Spearman's rank order correlation the value of rho was 0.65 ($p < 0.01$). This indicates a high level of agreement between children with type 1 diabetes and their parents with respect to the prominence of different hypoglycaemic symptoms. The dummy symptoms emerged with very low values for intensity and frequency for both children and their parents.

To assess whether some relationship might exist between the individual differences in the degree of intensity of symptoms perceived by the children and the same signs observed by their parents, a correlation was computed between the symptom scores of both groups using Spearman's rank correlation (Table 4.1.2). This analysis was applied only to the 43 children (and their parents) who were able to complete the symptom inventory. The symptoms of sweating, hunger, dizziness, headache, abdominal pain, irritability, being argumentative and being naughty correlated highly significantly (all $p < 0.001$, Table 2). Most symptoms reported by children and their parents showed significant correlations (range of rho corrected for ties 0.31-0.70, Table 4.1.2), and the only symptoms for which no significant correlations were noted were nausea, confusion, double vision and nightmares.

In summary, close agreement was observed between the reports of children with type 1 diabetes and their parents with respect to a) relative frequencies, and b) individual differences in intensities of symptoms of hypoglycaemia .

Symptoms of hypoglycaemia: Principal Components Analysis

In the next series of analyses we sought to discover the latent structure of hypoglycaemic symptoms in the reports of children with type 1 diabetes and their

parents; i.e. do the children and their parents report symptoms as coherent groups, and are these symptom groupings comparable between children and their parents ?

(1) Signs of hypoglycaemia observed by the parents.

The parental ratings of signs of hypoglycaemia observed in their children were subjected to principal components analysis followed by varimax rotation. The first unrotated principal component is 'general' in nature and is a means by which signs can be assessed as to whether they are in fact associated with a 'general hypoglycaemic factor'. Most symptoms demonstrated moderate to high loadings on this component (Table 4.1.3) which suggests that the symptoms presented in the questionnaire are related to the experience of hypoglycaemia.

After orthogonal varimax rotation, an examination of the scree slope indicated that a three factor solution offered the best description of the underlying symptom factors. Factor 1 had moderate to high loadings (i.e. ± 0.30 or greater) on symptoms (listed in descending order) of being argumentative, being aggressive, irritability, abdominal pain, confusion, poor concentration, tearfulness, bed-wetting, nightmares and headache. The nature of many of these symptoms indicates general behavioural disturbance. Factor 2 had moderate to high loadings for weakness, dizziness, sleepiness, sweating, slurred speech, trembling, pallor and convulsions. This factor would appear to comprise a mixture of neuroglycopenic and autonomic symptoms. Factor 3 had moderate to high loadings for blurred vision, double vision, headache, being aggressive, dizziness and slurred speech. The symptoms of nausea and hunger failed to load highly on any of the factors.

(2) Symptoms of hypoglycaemia reported by children with type 1 diabetes

The first unrotated principal component for each symptom again showed moderate

to high loadings for almost all symptoms. Application of the scree test to the unrotated principal components matrix suggested a two factor model (which accounted for 23.7% and 14.7% respectively, of the total variance). This model was subjected to an orthogonal varimax rotation and the result of this analysis is shown in Table 4.1.4. Factor 1 showed moderate to high loadings (given in descending order) for double vision, blurred vision, poor concentration, dizziness, confusion, weakness, sweating, hunger and trembling. These represent both neuroglycopenic and autonomic symptoms, which appeared to cluster together in this analysis. Factor 2 showed moderate to high loadings for being argumentative, irritability, being aggressive, being naughty and headache, suggesting general behavioural disturbance.

The Edinburgh Hypoglycaemia Scale for insulin-treated adult patients has proposed 11 key symptoms of hypoglycaemia which segregate into three distinct subgroups. Three of these symptoms (palpitations, odd behaviour, incoordination) were not included in our study because it was thought that the children might have difficulty in understanding the nature of these symptoms, and because they are not usually reported as hypoglycaemic symptoms by type 1 diabetic children (Macfarlane et al. 1989; Aman et al. 1989). In addition the terminology was modified to use 'sleepiness' instead of drowsiness, and 'trembling' rather than shaking. To compare directly the common symptoms of hypoglycaemia experienced by adults with type 1 diabetes (Deary et al. 1993b) with those of the type 1 diabetic children factor analysis was applied to the 8 remaining symptoms in the Edinburgh Hypoglycaemia Scale which had been reported by the subgroup of 43 children who were able to respond to the questionnaire. Three factors were extracted (Table 4.1.5) from this analysis. Factor 1 showed moderate to high loadings for hunger, confusion, sweating, slurred speech and trembling. This factor comprises a mixture of neuroglycopenic and autonomic symptoms. Factor 2 showed moderate to high loadings for nausea and headache. This may be interpreted as representing a non-

specific malaise factor. Factor 3 showed a high loading for trembling and a high negative loading for sleepiness. This bipolar factor appears to represent two apparently opposing states.

Table 4.1.1. Clinical details of children with type 1 diabetes.

	Children able to complete symptom profile (n = 43)		Children not completing symptom profile (n = 57)		Significance
	Median	Range	Median	Range	
Age (years)	11.4	3.0 - 16.0	9.0	1.5 - 14.0	p < 0.01
Age at diagnosis (years)	6.7	1.5 - 13.0	5.2	1.0 - 13.5	p < 0.05
Duration of diabetes (years)	4.5	0.3 - 12.0	4.1	0.5 - 13.0	NS
	Mean	SD	Mean	SD	
Insulin dose (unit/kg body weight)	1.0	0.3	0.9	0.3	NS
Total glycated haemoglobin (%)	10.5	1.4	10.3	1.4	NS

Table 4.1.2. Frequency and mean intensity of hypoglycaemic symptoms reported by the diabetic children and observed by their parents, and correlations (Spearman's rho) between the symptom scoring of the children and their parents.

Symptom	Frequency (%)		Intensity		Rho	
	Parent	Child	Parent	Child		
poor concentration	69	67	3.5	3.3	0.3	p<0.05
headache	47	64	2.9	2.7	0.6	p<0.01
nausea	33	19	1.7	1.5	0.1	ns
sweating	77	63	4.0	3.6	0.6	p<0.01
confusion	60	60	3.2	2.7	0.2	ns
trembling	64	72	3.4	3.6	0.4	p<0.01
sleepiness	67	67	3.6	3.3	0.5	p<0.01
tearfulness	74	42	4.0	2.0	0.4	p<0.01
tummy pain	43	45	2.5	2.2	0.6	p<0.01
irritability	73	42	4.0	2.3	0.5	p<0.01
dizziness	51	70	2.8	3.6	0.5	p<0.01
weakness	64	81	3.2	4.1	0.4	p<0.01
hunger	69	65	3.8	3.9	0.6	p<0.01
aggressive	64	51	3.8	2.9	0.5	p<0.01
blurred vision	19	45	1.6	2.1	0.4	p<0.05
convulsion	16	9	1.3	1.2	0.5	p<0.01
double vision	11	28	1.3	1.6	0.2	ns
argumentative	69	55	3.8	2.9	0.7	p<0.01
naughty	40	30	2.4	2.0	0.5	p<0.01
nightmares	20	7	1.5	1.1	0.2	ns
slurred speech	29	30	1.9	1.9	0.3	p<0.05
pallor *	88		5.3			
bed-wetting *	10		1.3			

* signs of hypoglycaemia observed by the parents of type 1 diabetic children

Table 4.1.3 Factor analysis of signs of hypoglycaemia observed by parents of type 1 diabetic children..

SYMPTOM	FIRST PRINCIPAL COMPONENT	FACTOR 1	FACTOR 2	FACTOR 3
Poor concentration	.581	.490	.295	.123
Tearfulness	.452	.449	.319	-.286
Tummy pain	.377	.532	.031	-.180
Irritability	.554	.628	-.008	.243
Argumentative	.575	.694	-.055	.265
Naughty	.528	.653	.044	.001
Nightmares	.362	.383	.222	-.220
Aggressive	.667	.644	.111	.395
Confusion	.579	.508	.234	.193
Bed wetting	.417	.472	.210	-.247
Trembling	.295	.023	.435	.094
Sleepiness	.393	.146	.572	-.181
Dizziness	.358	-.119	.593	.388
Weakness	.471	.021	.735	.111
Sweating	.418	.154	.562	-.092
Slurred speech	.412	.064	.479	.315
Convulsion	.230	-.002	.373	.059
Pallor	.360	.205	.388	-.080
Double vision	.295	.051	.167	.583
Blurred vision	.198	.015	-.014	.710
Headache	.519	.382	.156	.496
Nausea	.396	.235	.314	.123
Hunger	-.087	-.134	.021	.014

Table 4.1.4 Factor analysis of symptoms of hypoglycaemia reported by type 1 diabetic children

SYMPTOM	FIRST PRINCIPAL COMPONENT	FACTOR 1	FACTOR 2
Headache	.295	-.053	.598
Sweating	.461	.549	.014
Confusion	.609	.585	.223
Trembling	.405	.384	.156
Poor concentration	.690	.696	.205
Dizziness	.531	.595	.071
Weakness	.594	.569	.222
Hunger	.530	.451	.278
Blurred vision	.585	.726	-.024
Double vision	.632	.770	-.005
Slurred speech	.437	.576	-.066
Irritability	.478	.043	.782
Aggressive	.655	.278	.752
Argumentative	.442	-.083	.901
Naughty	.388	.005	.678
Sleepiness	.229	.134	.210
Tearfulness	.200	.088	.226
Tummy pain	-.066	-.137	.082

Table 4.1.5. Results of factor analysis applied to 8 symptoms of hypoglycaemia reported by children with type 1 diabetes that are common to adult patients with type 1 diabetes.

SYMPTOM	FIRST PRINCIPAL COMPONENT	FACTOR 1	FACTOR 2	FACTOR 3
Sweating	.67	.76	-.10	.16
Trembling	.43	.36	-.07	.66
Hunger	.66	.80	-.04	-.18
Confusion	.69	.63	.34	-.03
Slurred speech	.62	.52	.21	.35
Headache	.31	-.04	.84	.06
Nausea	.46	.13	.86	-.03
Sleepiness	.02	.25	-.07	-.86

Discussion

In the present study the symptoms of hypoglycaemia reported most frequently by the children with type 1 diabetes were weakness, trembling, dizziness, poor concentration and sleepiness. By comparison the parents of the children most frequently observed signs of sweating, tearfulness, irritability, poor concentration, being argumentative, and hunger. The parents also commonly observed pallor. However, when correlations between the symptoms of hypoglycaemia reported by children and those observed by their parents were examined, a general agreement emerged between the overall frequencies and individual differences in symptom intensities obtained from the parents and their children. Most symptoms correlated significantly (Table 4.1.2), contradicting the view that children are unable to recognise the symptom-complex of acute hypoglycaemia.

Factor analysis of the symptoms of hypoglycaemia in this study indicated that both parents and children identify a cluster of symptoms which represent a combination of autonomic and neuroglycopenic symptoms (Tables 4.1.3 and 4.1.4). The symptoms of trembling, sweating and hunger are usually classified as autonomic symptoms, while poor concentration, slurred speech and confusion are grouped as neuroglycopenic symptoms; physiological evidence exists to support this division (Hepburn, 1993). In diabetic adults, drowsiness (or sleepiness) usually indicates neuroglycopenia. Although the children did not report sleepiness with sufficient frequency for its inclusion in a symptom cluster, it was reported by their parents as an important indicator of hypoglycaemia, and it co-segregated with the autonomic/neuroglycopenic group of symptoms. The children's symptoms of blurred vision and double vision appeared to cluster with the autonomic/neuroglycopenic group of symptoms, which is consistent with observations reported previously in adults with type 1 diabetes (Hepburn et al. 1992;

Hepburn et al. 1991a).

For both the children with type 1 diabetes and their parents factor analysis classified weakness as an autonomic/neuroglycopenic symptom. This differs from factor analysis of hypoglycaemic symptoms in diabetic adults where weakness has been grouped with the non-specific symptoms of malaise (Hepburn et al. 1992), although in earlier studies weakness has been allocated both to autonomic and neuroglycopenic groups (Hepburn et al. 1991a). Pallor, observed by the parents, was allocated by factor analysis to the combined autonomic/neuroglycopenic group.

In the present study the clustering together of autonomic and neuroglycopenic symptoms was unexpected as adult type 1 diabetic patients are able to distinguish clearly between the two types of symptoms (Hepburn et al. 1992; Hepburn et al. 1991a; Deary et al. 1993b). It has been shown that the hormonal counterregulatory response to hypoglycaemia is more vigorous in non-diabetic, and in type 1 diabetic children compared with adults, with an epinephrine response of significantly greater magnitude occurring with less profound hypoglycaemia (Jones et al. 1991). Furthermore type 1 children with poor glycaemic control demonstrated a rise of symptom scores at plasma glucose concentrations that were within the normoglycaemic range (Jones et al. 1991). The most prominent symptom scores were alleged to be autonomic in nature when the childrens' responses were compared with those of adults with type 1 diabetes (Jones et al. 1991). A distinct cluster of autonomic symptoms might therefore have been anticipated in an analysis of hypoglycaemia symptomatology in children with type 1 diabetes. However, type 1 diabetic children develop abnormal cognitive function during mild hypoglycaemia, and appear to be much more sensitive to neuroglycopenia than adults (Ryan et al. 1990). The clustering together of autonomic and neuroglycopenic symptoms may either reflect very similar glycaemic thresholds for the activation of these groups of symptoms, or may result from a disruption in cognition so affecting the child's

perception of hypoglycaemia.

An important observation in the present study is the significance, which the parents of the children attached to a behavioural change in their child during hypoglycaemia. It has been reported that behavioural change, as an early feature of hypoglycaemia, occurs in only about 4% children with type 1 diabetes (Macfarlane et al. 1989), although others have noted that irritability is more frequently reported during hypoglycaemia (Aman et al. 1989). Behavioural changes have received little attention as premonitory signs of a falling blood glucose. In the present study behavioural change was common, and factor analysis indicated it accounted for a significant amount of the total variance in each analysis. For both parents and children this sub-group of hypoglycaemic symptoms associated with behavioural change consisted of irritability, being aggressive and being argumentative. Of the symptoms reported by the children headache and being naughty clustered with this group, while the parental observations included tearfulness, tummy pain, poor concentration, headache, nightmares, bed wetting and slurred speech. Some symptoms segregated into more than one factor, which may be related to the relatively small size of the study population. A third factor, formed within the factor analysis of the parents' observations, included the symptoms of blurred vision, double vision, headache, dizziness and being aggressive. This factor appears to suggest that the parents were observing a particular pattern of neurological dysfunction when their child was hypoglycaemic but this interpretation is open to speculation.

The final analysis was to use the Edinburgh Hypoglycaemia Scale (Deary et al. 1993b) to compare the symptoms of type 1 diabetic children directly with those of type 1 diabetic adults reported in previous studies from our department (Table 4.1.5). This analysis also demonstrated that the children did not differentiate between autonomic and neuroglycopenic symptoms; instead they tended to combine

these groups as a distinct cluster of symptoms. The second factor from this analysis showed very high loadings for nausea and headache and clearly represents the non-specific malaise factor reported by Deary *et al.* (1993). This second factor is also reported commonly by adult type 1 diabetic patients during hypoglycaemia. Thus, the present study, would appear to confirm the existence of a hypoglycaemia-associated non-specific malaise factor within a group of ambulant, type 1 diabetic children. The third factor extracted from this final analysis appears to consist of two apparently opposing states, namely that of trembling and sleepiness.

In common with other studies of type 1 diabetic children (Stern, 1995; Goldstein *et al.* 1981; Bergada *et al.* 1989), a relatively high rate of severe hypoglycaemia was recorded in the present survey. Almost half of this unselected cohort of type 1 diabetic children had experienced severe hypoglycaemia at some time. As the present survey employed retrospective estimates it can not provide an accurate measure of the true frequency of severe hypoglycaemia within this age group. However, because a strict definition of severe hypoglycaemia was used in the present survey, the estimated frequency is likely to be reasonably accurate as most parents are unlikely to forget the traumatic experience of a severe episode. In a recent survey Limbert *et al.* studied 74 type 1 diabetic children and adolescents prospectively over a 12 month period and reported that 44 (59%) of their patients experienced at least one episode of severe hypoglycaemic episode and almost all had experienced symptomatic hypoglycaemia.

In adult type 1 diabetic patients, increased risk of severe hypoglycaemia is associated with a past history of severe hypoglycaemia, increasing age and duration of diabetes, strict glycaemic control, and loss of symptomatic awareness of hypoglycaemia (MacLeod *et al.* 1993). Patients taking large doses of insulin, and who have a low glycated haemoglobin concentration, experience a higher frequency of severe hypoglycaemia (The Diabetes Control and Complications Trial Research

Group, 1991). In the present study of type 1 diabetic children a positive association was observed between the frequency of previous severe hypoglycaemia and an early age of diagnosis. A significant association was observed between the frequency of severe hypoglycaemia and the parents' self-reported ability to recognise the symptom-complex of hypoglycaemia. Interestingly, the ability of the parental group to detect hypoglycaemia in the children improved with time suggesting that this may indicate deficiencies in the parents' knowledge of the manifestations of hypoglycaemia early in the course of treatment with insulin. The question of whether children develop impaired awareness of hypoglycaemia remains undetermined.

In summary:-

This study has documented the symptoms of hypoglycaemia most frequently reported by type 1 diabetic children and observed by their parents, and has shown general agreement between the parents and their children in the reporting of symptoms of hypoglycaemia. Type 1 diabetic children and *type 1 diabetic* adults clearly differ in their reporting of symptoms of hypoglycaemia, and factor analysis of the parents' and the childrens' reported symptoms has identified two predominant symptom clusters: a combined autonomic/neuroglycopenic group of symptoms and a behavioural group of symptoms. This information may be of value when educating newly diagnosed type 1 diabetic children and their parents about hypoglycaemia.

PART V

CONCLUSIONS AND FUTURE RESEARCH

In Chapter 2 of this thesis I reviewed the published literature on the effects of both acute and chronic insulin-induced hypoglycaemia on cognitive function. In general it can be inferred from this literature that almost all major modalities of cognitive function (motor, verbal, memory, attention and executive functions) can become significantly impaired during acute moderate hypoglycaemia (approx. 2.4-3.0 mmol/l). However, much of this data relied on cognitive tasks that are non-specific, usually accessing more than one modality of cognitive function. It could not, for instance, be inferred that more fundamental cognitive processes were being significantly affected by hypoglycaemia, nor could the outcome of such studies be translated directly into the everyday life of individuals. This distinction is important because in cognitive terms the brain is not a single system that either functions normally or abnormally when subjected to certain stresses. If the brain were able to re-direct glucose or alternative substrates to more essential cognitive processes during the physiological stress of hypoglycaemia it could maintain normal metabolism and hence these cognitive functions would be preserved. If this were true, it would imply that fundamental cognitive processes would be unaffected by moderate hypoglycaemia and that an individual exposed to this physiological state could function normally. In order to address these issues studies needed to be designed that would examine the impact of moderate hypoglycaemia on more fundamental cognitive processes, and on the performance of everyday activities (e.g. driving).

Hypoglycaemia and cognitive function: Sensory information processing

The first and principal aim of this thesis was to examine the impact of acute moderate hypoglycaemia on two essential cognitive processes, namely *visual* and *auditory information processing*. These two aspects of sensory perception were chosen because of their importance to humans engaged in daily activities, and because tests have been developed which provide an accurate index of these

processes. Non-diabetic subjects rather than subjects with type 1 diabetes were chosen for these studies because, in general, fewer variables influence this population that might effect cognitive performance during hypoglycaemia (e.g. previous experience of hypoglycaemia and glycaemic control). The impact of hypoglycaemia per se on these tasks could, therefore, be more clearly documented.

Studies 1 and 2

The outcome of Studies 1 and 2 suggest that moderate hypoglycaemia does have a significant disruptive effect on basic sensory information processing (visual and auditory information processing). All three tasks of visual information processing (Inspection Time, Visual Change and Visual Movement Detection) and two of the four tasks of auditory information processing (Temporal Order and Single-Tone Loudness discrimination) showed significant disruptions during moderate hypoglycaemia. This indicates that essential brain functions are *not* protected from the disruptive effect of moderate hypoglycaemia.

Studies 1 and 2 provide evidence of significant disruption to fundamental cognitive processes in non-diabetic individuals during acute moderate hypoglycaemia. Because humans make use of auditory and visual perception during most daily activities, the impairment of these processes may translate to impaired performance on these activities. An even greater impact may be seen on those activities that rely predominantly on visual perception because of the clearer effects of hypoglycaemia found on visual information processing tasks and the added effect of disrupted contrast sensitivity. However, before such a conclusion can be drawn studies need to be conducted that will directly assess performance during hypoglycaemia on different everyday activities. Cox et al. (1993) demonstrated significant disruption to driving ability in individuals with type 1 diabetes during moderate hypoglycaemia which indirectly supports the findings of Studies 1 and 2. However, the studies of

this group on driving are isolated investigations and more studies on other activities need to be completed before the scientific community can be certain of the importance of this data.

It should be recognised that individuals with type 1 diabetes form a much more heterogeneous population than non-diabetic individuals. Glycaemic control, antecedent hypoglycaemia and hypoglycaemia unawareness have all been shown to affect cognitive performance during acute hypoglycaemia. It is possible that individuals with type 1 diabetes might be protected from the disruptive effects of hypoglycaemia on visual and auditory information processing. This is a controversial area of research, with some type 1 individuals showing preserved function on some cognitive tasks (in general those individuals with a short duration of disease and near-normal glycaemic control) during hypoglycaemia, while others (e.g. those with poor glycaemic control or hypoglycaemia unawareness) exhibit a greater deterioration of cognitive function. Further studies designed to examine the impact of moderate hypoglycaemia on visual and auditory information processing in individuals with type 1 diabetes would help to clarify this issue.

Hypoglycaemia: Effects on mood and emotion

The second major aim of this thesis was to examine the effect of moderate hypoglycaemia on measures of anger, mood and appraisal. The literature on the effect of hypoglycaemia on human emotions and mood is limited. A review of that literature suggests that both in non-diabetic individuals and in people with type 1 diabetes hypoglycaemia leads to a negative mood state. There was also evidence to suggest that hypoglycaemia might result in some non-diabetic individuals displaying aggressive or violent behaviour. In Study 3 it was hypothesised that hypoglycaemia might lead to an increase in anger-state, and in Study 4 it was hypothesised that hypoglycaemia, through the induction of a negative mood state, would result in the

expression of negative emotions, and negative appraisals of a life event. These studies served two purposes. On the one hand they provided further useful information on the effect of hypoglycaemia on human emotion and mood, and on the other they provided further validation of the usefulness of hypoglycaemia as a model for inducing the theoretically important state of tense-tiredness.

Studies 3 and 4

In Study 3, individuals asked to rate feelings of anger in the non-confrontational and artificial setting of the laboratory, reported a small, but significant, increase in anger-state during acute hypoglycaemia. Considerable inter-individual variability was observed with a marked increase in anger-state in a few individuals. It is possible that the increase in anger shown by these individuals during hypoglycaemia may lead to aggressive behaviour depending on the provocation or other aspect of the situation. No correlations were found between the increase in anger-state and measures of anger-trait and general anger-expression. However, the number of subjects in the study was probably insufficient to exclude a possible association. Hypoglycaemia-associated anger is a poorly understood phenomenon but one which may adversely effect an individual's social and work relationships and may explain why some subjects with type 1 diabetes refuse assistance with the treatment of hypoglycaemia when it is proffered.

In Study 4, hypoglycaemia was demonstrated to induce a negative mood state in non-diabetic individuals, which was characterised by decreased happiness and increased tension. Moreover, these individuals reported more negative appraisals of a life-event. Importantly, personality dimensions remained stable during experimental hypoglycaemia indicating that the effects on mood and emotion were situational in nature i.e. induced by the experimental condition. As with the increase in anger-state such negative moods and emotions might have an adverse impact on

an individual's social and work interactions.

Taken together with previous studies of mood and emotion during hypoglycaemia it can be concluded from the findings of Studies 3 and 4 that acute hypoglycaemia has a negative impact on these aspects of human behaviour. Whether these findings, for the reasons stated earlier, can be translated directly to people with type 1 diabetes is not certain. However those investigators who have studied individuals with type 1 diabetes have reported similar findings (Gold et al. 1995c, Hepburn et al. 1995, Gonder-Frederick et al. 1989). Two other important questions arise from this work that could be addressed in future studies. Firstly, what determines the marked individual variability noted in the change in mood and emotion during hypoglycaemia? Greater comprehension of this may enable more specific therapy to be directed to such individuals, and may provide useful information for their families and work colleagues. Secondly, what is the impact of recurrent hypoglycaemia on mood? It is possible that recurrent hypoglycaemia may have a potential psychomorbidity and, given the current drive to achieve near-normalisation of glycaemic control in type 1 diabetes, this could have important implications for the subsequent mental health of individuals with type 1 diabetes.

Hypoglycaemia: Symptomatic response to hypoglycaemia

In the final section of this thesis the symptomatic response to hypoglycaemia was examined in two different situations: the pattern of symptoms reported during experimental hypoglycaemia induced by either the hyperinsulinaemic glucose clamp or the insulin-infusion technique in type 1 diabetic and non-diabetic individuals (Study 5), and the symptoms reported by type 1 diabetic children and observed by their parents (Study 6).

Study 5

In Study 5, data was pooled from the studies in this thesis and from others conducted in Edinburgh during the last 10 years, to show that individuals with type 1 diabetes and non-diabetic individuals reported the same pattern of symptoms during hypoglycaemia and that this pattern did not differ when hypoglycaemia was induced using either the hyperinsulinaemic glucose clamp or the insulin-infusion techniques. From this large data set the symptoms which were reported with the greatest frequency and intensity during experimental hypoglycaemia were also extracted and may usefully lead to the refinement of a symptom questionnaire for hypoglycaemia, with development of a questionnaire specifically for experimental hypoglycaemia. The importance of this relates mostly to establishing a degree of uniformity between centres such that studies between different research groups can be more readily compared.

Study 6

In Study 6 it was shown that children with type 1 diabetes clearly differed from adults in their reporting of hypoglycaemia symptoms with much more emphasis being placed on behavioural changes. However, it is also evident from the literature that little emphasis has been placed on determining the behavioural effects of acute hypoglycaemia in the adult population with type 1 diabetes. Taken together with the findings of Studies 3 and 4, which suggested acute hypoglycaemia may have significant effects on mood and emotion in people with type 1 diabetes, it is possible that the differences noted in Study 6 were exaggerated because adults with type 1 diabetes are rarely asked about, or know to report, behavioural changes during acute hypoglycaemia. The findings of Study 6 do have practical implications for the management of children with type 1 diabetes and may be of value when educating

newly diagnosed children and their parents about hypoglycaemia. Future research aimed at exploring specific effects of hypoglycaemia on the behaviour of type 1 diabetic children could provide further useful information for the parents who provide their daily care.

Summary

The original studies described in this thesis provide evidence that:

- (1) Acute insulin-induced hypoglycaemia provokes a significant impairment of visual and auditory information processing in non-diabetic individuals.
- (2) That acute insulin-induced hypoglycaemia in non-diabetic individuals can induce a behavioural state characterised by increased anger, increased tension and decreased happiness, and that this state leads to the reporting of more negative appraisals of a life event.
- (3) Experimental hypoglycaemia tends to generate the same pattern of symptoms in individuals with or without type 1 diabetes irrespective of the method used to generate hypoglycaemia.
- (4) Children with type 1 diabetes who experience hypoglycaemia in the community tend to express more signs of behavioural change than do adults with type 1 diabetes.

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APPENDIX

Abstracts

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